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CARCINOGENESIS ABSTRACTS

National Cancer Institute

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CARCINOGENESIS ABSTRACTS

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PREFACE

Carcinogenesis Abstracts is a publication of the National Cancer Institute. The journal serves as a vehicle through which current documentation of carcinogenesis research highlights are compiled, condensed, and disseminated on a regular basis. It represents an integral part of the Institute's program of fostering and supporting coordinated research into cancer etiology. Issues of *Carcinogenesis Abstracts* normally contain three-hundred abstracts and three-hundred citations (unaccompanied by corresponding abstracts). Abstracts and citations refer to the current scientific literature that describes the most significant carcinogenesis research carried on at the National Cancer Institute, other governmental agencies, and private institutions. *Carcinogenesis Abstracts* is intended to be a highly useful current awareness tool for scientists engaged in carcinogenesis research or related areas. The great number and diversity of publications relevant to carcinogenesis make imperative the availability of this service to investigators whose work requires that they keep abreast with current developments in the field.

Carcinogenesis Abstracts is normally published monthly. Volume XII covers the scientific literature published from Jan 1974 through Dec 1974. To increase the usefulness of *Carcinogenesis Abstracts*, Volume XII, a Wiswesser Line Notation index and a Chemical Abstracts Service Registry Number index have been provided. These indexes reference compounds described in abstracted articles. A cumulative subject, author, CAS Registry Number, and Wiswesser Line Notation index for Volume XII will be published shortly after the final regular issue.

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NOTE

Journal names are abbreviated according to the list of abbreviations used by *Index Medicus*. For journals not covered by *Index Medicus*, the abbreviations (with some modifications) found in *World Medical Periodicals*, 3rd Edition, are used.

LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	It.	Italian
Ar.	Arabic	Jap.	Japanese
Bul.	Bulgarian	Kor.	Korean
Ch.	Chinese	Latv.	Latvian
Cz.	Czech	Lith.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
E.	English	Por.	Portuguese
Eston.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fl.	Flemish	Ser.	Serbo-Croatian
Fr.	French	Sl.	Slovak
Ger.	German	Sp.	Spanish
Gr.	Greek	Sw.	Swedish
Heb.	Hebrew	Th.	Thai
Hun.	Hungarian	Turk.	Turkish
Ic.	Icelandic	Uk.	Ukrainian
In.	Indonesian	Viet.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

ACTH	adrenocorticotropic hormone	mg	milligram(s)
ADP	adenosine diphosphate	min	minute(s)
AMP	adenosine monophosphate	ml	milliliter(s)
ATP	adenosine triphosphate	mm	millimeter(s)
C	degrees centigrade	MTD	maximum tolerated dose
cm	centimeter(s)	ng	nanogram (10^{-9})
CNS	central nervous system	pg	picogram (10^{-12})
cpm	counts per minute	p.o.	orally
DNA	deoxyribonucleic acid	ppm	parts per million
e.g.	for example	r	Roentgen
g	gram(s)	RBC	red blood cells (erythrocytes), red blood count
μg	microgram(s)		
hr	hour(s)	resp.	respectively
i.m.	intramuscular	Rev.	review (only in citations)
i.p.	intraperitoneal	RNA	ribonucleic acid
IU	international unit(s)	s.c.	subcutaneous
i.v.	intravenous	sec	second(s)
kg	kilogram(s)	U	unit(s)
LD$_{50}$	median lethal dose(s)	UV	ultraviolet
m	meter(s)	WBC	white blood cells (leukocytes), white blood count
M	molar		
mEq	milliequivalent(s)	wk	week(s)
mM	millimolar	wt	weight(s)
μM	micromolar	yr	year(s)
mC,μC	milli-,microcurie(s)		

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001 SPECIAL REPORT: CHEMICAL CARCINOGENESIS.
(E.) Ryser, H. J. P. (Boston Univ. Sch.
ed., Mass.). *Cancer J Clin* 24(6):351-360, 1974.

he various classes of carcinogens such as the poly-
yclic aromatic hydrocarbons (benzo[a]pyrene), the
romatic amines (butter yellow, naphthylamine and
-acetylaminofluorene), the alkylating agents (epox-
des and lactones), nitrosamines, aflatoxins, natural
arcinogens (cycasin), chemotherapeutic agents and
eavy metals are tabulated. Dose-dependency, duration
f carcinogenesis induction, genetic transmission
f the carcinogenic change, chemical promoters (co-
arcinogens) and effects of growth-stimulating
actors are reviewed. The importance of interaction
f the metabolically activated carcinogen with
NA is discussed with regard to various theories
f carcinogenesis. The cellular environment bound
y the membrane and extracellular microenvironment
oupled with a variety of other systemic host
actors, including immunological ones, make the pro-
osal of a unifying theory of carcinogenesis diffi-
ult. A theory of carcinogenesis with multiple
teps including an initiation phase, a latent period,
nd the final appearance of irreversible malignant
rowth is presented. While changes in the initia-
ion phase may be irreversible they are not neces-
arily committed to complete malignant transformation
uring the latent period. It is concluded that
hemical carcinogens are the principal causative
gents of human cancer. (33 references)

002 AN APPROACH TO THE EVALUATION OF THE CAR-
CINOGENICITY OF DRUGS. *(E.)* Terracini,
. (Inst. Pathol. Anat., Univ. Turin, Italy). *Proc
ifth Int Symp Biol Characterization Hum Tumors,
ologna, April 4-6, 1973* 1:125-132, 1974.

he carcinogenicity of drugs used to treat neoplastic
nd non-neoplastic disease in man is reviewed. The
nly authenticated cases are the trivalent arseni-
als used to treat psoriasis, naphthylamine nitro-
en mustard and diethyl stilbestrol. Chronic immu-
osuppression in organ transplant recipients is
onsidered in another category because of complex
ffects of the transplant as well as the drug therapy.
everal drugs used to treat human disease have been
tudied in animals in which, under certain conditions,
hey are carcinogenic. Only a few of the poten-
ially carcinogenic drugs have been thus studied.
our drugs are selected for more detailed review:
etronidazole, NFTA (a nitrofuryl thiazolylace-
amide analog), griseofulvin and isonicotinic acid
ydrazide. From these animal data, criteria are
resented for extrapolating potential carcinogeni-
ity of drugs to man. (61 references)

003 THE PROBLEM OF SPECIES VARIATIONS. *(E.)*
Smith, R. L. (St. Mary's Hosp. Med. Sch.,
ondon, England). *Ann Nutr Aliment* 28(4):335-349,
974.

major factor that determines interspecies varia-
ions in response to toxic chemicals is species differences in metabolism. Species differences in the pattern of metabolism, which are reviewed, commonly arise because of competing reactions, the occurrence of metabolic defects, and the occurrence of unusual metabolic reactions in some species. The extent to which hydroxylation of aniline occurs in the ortho or para positions varies with species; hydroxylation in the ortho position predominates in the dog, cat, and ferret, while p-hydroxylation predominates in rodents and in the rabbit. The dog lacks the capacity to carry out arylamine acetylation, the cat lacks the capacity to form glucuronide, while the rat is defective in *N*-hydroxylation of aliphatic amines. A number of metabolic reactions appear to be restricted to man and other primate species. This can be illustrated by considering the metabolic conjugation of phenylacetic acid. In man and monkey species, phenylacetic acid is largely conjugated with glutamine, whereas glycine is utilized in subprimates. Therefore, in this situation, a metabolic model species for man must be found among primate species. (19 references)

6004 ARYL HYDROCARBON HYDROXYLASE: REGULATION AND ROLE IN POLYCYCLIC HYDROCARBON ACTION.
(E.) Gelboin, H. V. (Natl. Cancer Inst., Bethesda, Md.), F. J. Wiebel and N. Kinoshita. *Biochem Dis* 4(Part A):309-351, 1974.

The role of the microsomal enzyme system in both the detoxification and activation of the polycyclic hydrocarbons is reviewed, as are current studies on the biologic regulation of the enzyme system. Five recently developed lines of evidence suggest a role for the microsomal hydroxylase system in the toxic and carcinogenic actions of the polycyclic hydrocarbons: the aryl hydrocarbon hydroxylase (AHH) enzyme catalyzes the formation of DNA-polycyclic hydrocarbon and protein-polycyclic hydrocarbon complexes; the cellular AHH level parallels cell susceptibility to polycyclic hydrocarbon toxicity; inhibitors of the AHH system prevent polycyclic hydrocarbon toxicity in hamster embryo cells; inhibitors of the AHH system inhibit dimethylbenz(a)anthracene (DMBA) tumorigenesis in mouse skin; and inhibitors of the AHH system reduce the covalent binding of DMBA to DNA, RNA, and mouse skin protein. The mechanism of AHH induction has been studied in cell culture systems which permit study of the molecular mechanism involved in the induction process. The results indicate that the induction process involves stabilization of the enzyme by the inducer-substrate of the enzyme system and the early production and accumulation of an induction-specific RNA followed by the independent synthesis of protein. Maximum induction can, however, occur when the synthesis of rRNA and its appearance in the cytoplasm are largely suppressed, which indicates that the induction specific RNA for AHH induction is located in the heterodisperse "M-RNA" region. Studies with cell hybrids suggest a closely coupled control mechanism for the inducibility and the basal level of AHH activity. Further analysis of hybrid cells may elucidate the genetic regulation of the components of this enzyme complex and may permit the study of carcinogen, xenobiotic, and drug metabolism and their modes of action. (68 references)

6005 THE BIOLOGICAL EFFECTS OF ASBESTOS. *(E.)* McCullagh, S. F. (James Hardie Coy Pty Ltd., Granville, Australia). *Med J Aust* 2(2):45-49, 1974.

Definition of and discrimination between pulmonary asbestosis and pleural asbestosis are presented the former condition being more specifically related to occupational asbestos exposure. The relation of lung carcinogenicity to physical (surface) characteristics of asbestos compared to chemical characteristics of asbestos is discussed together with the cocarcinogenic effects of tobacco smoking. The close causal relation of mesothelioma to pleural asbestosis is also discussed. The monitoring of atmospheric asbestos levels in asbestos-handling plants is reveiwed and compared with levels in the general environment (drinking water, urban streets). (51 references)

6006 BIOLOGICAL ASPECTS OF CHEMICAL CARCINOGENESIS: SUMMARY AND PREDICTIONS. *(E.)* Saffiotti, U. (Natl. Cancer Inst., Bethesda, Md.). *Biochem Dis* 4(Part B):729-746, 1974.

The biological aspects of a symposium on chemical carcinogenesis are summarized. The discussion is organized around the three phases of the process of carcinogenesis: 1) events occurring before the critical phenomena of transformation; 2) events occurring during and around the time of transformation; and 3) events occurring after transformation has been accomplished and cells grow and establish themselves as a recognizable tumor. The discovery of the potential of *in vitro* short-term models for the study of the key events of carcinogenesis is considered a major step in assessing the central events of the carcinogenic process. The development of the new *in vitro* methods is beginning to allow researchers to study the interactions of different types of carcinogenic effects directly on the target cells. The study of hormonal effects in carcinogenesis and particularly the possibility of manipulating hormonal factors *in vivo* and *in vitro* in model systems for carcinogenesis was also discussed. Major advances in cell biology that were discussed for their potential usefulness in carcinogenesis research include the model for cell hybridization through fusion and the role of DNA replication in carcinogenesis. Both the control mechanisms that occur before the target interaction of a carcinogen (particularly the enzyme systems that regulate the balance of carcinogen detoxification and activation) and those that control the spread of established cancer cells (particularly the immunologic systems) were discussed. The symposium concentrated almost exclusively on data relating to the interaction of carcinogens with the genetic material in the cells. It is concluded that the development and study of models based on a single cell population should be continued but that they should be related to larger orders of organization; tissues, organs, and whole organisms so that all the main steps that lead from the entry of a carcinogen into the human body to the development of cancer can be identified and inhibited. (56 references)

6007 CELL ANTIGEN CHANGES DURING CHEMICAL CARCINOGENESIS. *(E.)* Baldwin, R. W. (Cancer Res. Campaign Labs., Univ. Nottingham, England). *Biochem Dis* 4(Part B):691-699, 1974.

Studies on the immunology of chemically induced rat tumors, particularly aminoazo dye-induced hepatomas and methylcholanthrene-induced sarcomas, which have special relevance to the study of chemical carcinogens, is reviewed. The individually specific rejection antigens are the most distinctive class of neoantigens associated with chemically induced tumors. They can be used to characterize *in vitro* transformed cells; detection of these antigens may, therefore, provide an important parameter for evaluating transformation. Recent developments in radioimmunoassay techniques will make possible precise analysis of the amount of antigen expressed on transformed cells so that tumor-specific antigens will provide specific and quantitative markers for studies on the extent of cellular derangement induced by a particular carcinogen. Another class of antigens that has been detected on carcinogen-induced rat tumors are re-expressed embryonic components. Studies indicate fairly clearly that these embryonic antigens differ from the tumor-specific antigens. The frequency of embryonic antigen expression in chemically-induced tumors has yet to be evaluated, so it cannot yet be stated that this event is necessarily concomitant with neoplastic transformation. It is concluded that the ultimate objective of such studies is to develop immunologic procedures for defining changes in cell surface architecture following transformation by chemical carcinogens. The use of specific immunohistologic labels should provide methods for ultrastructure studies of tumor antigen localization while developments in the biochemistry of tumor antigens will eventually lead to the precise characterization of these tumor components. (33 references)

6008 MOLECULAR MECHANISMS IN CHEMICAL CARCINOGENESIS. *(E.)* Magee, P. N. (No affiliation). *Recent Results Cancer Res* 44:2-8, 1974.

It is now firmly established that most, perhaps all, chemical carcinogens are electrophilic reactants or become converted into such reactants in the body. These so-called ultimate carcinogens react with nucleophilic centers in nucleic acids, proteins, and probably other cellular components. An increasing number of them have been shown to be mutagenic. In view of the complexity of the various intracellular targets for carcinogens, it is difficult to see how further study of carcinogen-macromolecule interaction can lead to precise conclusions on the molecular mechanism of malignant transformation. Recent advances in understanding of viral carcinogenesis and the renewed interest in possible activation of latent oncogenic viruses or oncogenes suggest that investigations of chemical carcinogenesis should be directed toward its possible interrelationship with viral carcinogenesis at the molecular level. (64 references)

6009 AROMATIC HYDROCARBON-PRODUCED TUMORIGENESIS AND THE GENETIC DIFFERENCES IN ARYL HYDROCARBON HYDROXYLASE.

(E.) Nebert, D. W. (Natl. Inst. Child.Health Hum. Dev., Bethesda, Md.), W. F. Benedict and R. E. Kouri. *Biochem Dis* 4(Part A):271-288, 1974.

The correlation between genetically controlled differences in mono-oxygenase activity and the susceptibility to tumorigenesis is discussed. Tumor susceptibility among littermates can be evaluated in mice in which the presence or absence of aryl hydrocarbon hydroxylase is expressed in their tissues. This model eliminated the problems posed by such nonspecific phenomena as characteristic mouse strain differences involving immunology, latent viral infections, nutrition, hormones, responses to stress, and levels of other enzymes. The susceptibility to tumorigenesis produced by the s.c. administration of 3-methylcholanthrene is highly correlated with the genetically mediated presence of aryl hydrocarbon hydroxylase induction in 14 strains of mice. There was no such relationship between inducible hydroxylase activity and s.c. treatment with 7,12-dimethylbenz(a)anthracene, i.p. administration of benzo(a)pyrene, or topical application of 7,12-dimethylbenz(a) anthracene or benzo(a)pyrene and promotion by phorbol ester. This genetically mediated difference in the activity of aryl hydrocarbon hydroxylase is offered as an experimental model for understanding chemical carcinogenesis. (40 references)

6010 MAMMALIAN ARYL HYDROCARBON HYDROXYLASES IN CELL CULTURES: MECHANISM OF INDUCTION AND ROLE IN CARCINOGENESIS.

(E.) Wiebel, F. J. (Natl. Cancer Inst., Bethesda, Md.), J. P. Whitlock and H. V. Gelboin. In: *Survival in Toxic Environments* edited by M. A. Q. Khan and J. P. Bederka, Jr. (New York: Academic Press, Inc., 1974) pp.261-293.

Studies of the existence and distribution of different forms of aryl hydrocarbon hydroxylases in experimental animals are described and the mechanism of induction in mammalian cells in culture is examined. The role of aryl hydrocarbon hydroxylases in the biological effect of polycyclic hydrocarbons is also considered. Hydroxylase induction was studied in a variety of cell cultures leading to the conclusion that induction requires continuous protein synthesis and transitory RNA synthesis. The synthesis, transfer, and functioning of induction-specific RNA is independent of concurrent ribosomal RNA synthesis. Enzyme activity may also be regulated at the post-transcriptional level. The relative importance of transcriptional and post-transcriptional control mechanisms varies between different cell types. Using the synthetic flavonoid, 7,8-benzoflavone, as a probe, at least two forms of aryl hydrocarbon hydroxylase could be distinguished. The relative activities of these two forms vary with age, sex, state of induction, nutrition and tissue of test animals. Observations which indicate that microsomal oxygenases convert polycyclic hydrocarbons not only to inactive metabolites, but also to derivatives which are toxic and carcinogenic are: 1) the covalent binding of polycyclic hydrocarbons to DNA is catalyzed by microsomal enzymes; 2) inhibition of enzyme activity by 7,8-benzoflavone inhibits polycyclic hydrocarbon-induced cytotoxicity; and 3) inhibition of enzyme activity inhibits cutaneous tumorigenesis by 7,12-dimethylbenz[a]anthracene, but not by benzo[a]pyrene. The role which the aryl hydrocarbon hydroxylases play in polycyclic hydrocarbon carcinogenesis may differ for individual hydrocarbons. (57 references)

6011 INTERACTION OF THE CARCINOGEN 7-METHYLBENZ(A)ANTHRACENE WITH DNA OF MAMMALIAN CELLS.

(E.) Brookes, P. (R. Cancer Hosp., London, England), W. M. Baird and A. Dipple. *Biochem Dis* 4(Part A):149-157, 1974.

The mechanism of interaction between cellular DNA and 7-methylbenz(a)anthracene (7MBA) was investigated using hydrocarbon-bound DNA derived from cultured rodent embryo cells. The results of such studies indicate that metabolism is required for the binding of carcinogenic hydrocarbons to cellular macromolecules. The relevance of this binding to the initiation of carcinogenesis and the nature of the essential cellular receptors is at this time unclear. A method for the isolation of the products of the hydrocarbon-DNA reaction is currently being developed; this involves fractionation using water: methanol gradient elution on LH20 Sephadex columns. Models are being developed to reproduce these products *in vitro*: in one such system, mouse embryo cells in culture are treated for 4-24 hr with (^{3}H)-7MBA-epoxide and the DNA subsequently isolated; in another system, an aqueous-ethanol solution of DNA is treated with iodine plus (^{3}H)-7MBA. Hopefully, these techniques will eventually allow the complete identification of the products of the hydrocarbon-DNA reaction and perhaps a better understanding of the process of chemical carcinogenesis at the molecular level. (25 references)

6012 HYPOTHESIS CONCERNING THE CARCINOGENIC ACTIVITY OF BENZ(a)ANTHRACENES.

(E.) Newman, M. S. (Dep. Chem., Ohio State Univ., Columbus). *Biochem Dis* 4(Part A):177-181, 1974.

A hypothesis for the role of benz(a)anthracenes in initiating cancer is proposed based on experiments that indicated that the 5-position is the important position in the metabolism of these compounds leading to cancer. It is suggested that benz(a)anthracenes may be metabolized in two ways: 1) metabolism at position 7 which results in a reaction series that leads to non-carcinogenic processes; and 2) metabolism at position 5 which results in a reaction series that leads to cancer. In the parent hydrocarbon, the metabolism leading to deactivation (from the carcinogenic point of view) at position 7 must be considerably more rapid than the interaction at position 5, which leads to the production of cancer. Similarly, in other benz(a)anthracenes in which the 7-position is available for attack, it is argued that the metabolism leading to deactivation is more rapid than that leading to the production of cancer. It is also postulated that the fact that 7,12-dimethylbenz(a)anthracene and 6,8,12-trimethylbenzanthracene are appreciably more active than the 7-methyl- and 6,8-dimethylbenz(a)anthracenes, resp., is due to the steric effect of the 12-methyl group which causes the whole molecule to be strained and more reactive toward external reactants. It is suggested that these concepts may be extended to compounds in the dibenz(a,h)anthracene and benz(a)pyrene series. (18 references)

6013 ADENOSIS AND ADENOCARCINOMA OF THE VAGINA IN YOUNG WOMEN: A REVIEW. *(E.)* Yon, Jr., J. L. (Univ. Miami Sch. Med., Fla.), M. H. Lutz, R. E. Girtanner and H. E. Averette. *Gynecol Oncol* 2(4):508-517, 1974.

Because of the recent increased incidence of adenosis and adenocarcinoma of the vagina in young women, presumably related to maternal ingestion of estrogenic compounds during pregnancy, a review is presented and four new cases of vaginal adenocarcinoma seen since June 1972, one in a seven-yr-old, are reported. In one young woman and in the case of the seven-yr-old, there had been maternal ingestion of Diethylstilbestrol; in two cases, the mothers denied ingestion of estrogenic compounds. It is concluded that nonsteroidal synthetic estrogens and possibly all estrogenic substances are contraindicated during pregnancy. (20 references)

6014 ON THE ORIGIN OF RNA TUMOR VIRUSES. RELATIONSHIPS AMONG RNA TUMOR VIRUSES AND BETWEEN RNA TUMOR VIRUSES AND CELLS. IMPLICATIONS FOR THE ORIGIN OF VIRUSES, THE EVOLUTION OF VERTEBRATES, AND CELLULAR GENETIC SYSTEMS. *(E.)* Temin, H. M. (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison). *Annu Rev Genet* 8:155-177, 1974.

Relationships among different RNA tumor viruses and between RNA tumor viruses and cells have implications for the origin of viruses, the evolution of vertebrates, and cellular genetic systems. The 14 groups of viruses which are referred to consist of avian leukosis-sarcoma, mouse C-type, feline C-type, rat C-type, hamster C-type, simian C-type, RD-114-like C-type, murine, feline, and simian sarcoma, viper C-type, mouse mammary tumor, Mason-Pfizer monkey, visna, synctium-forming, and reticuloendothelial viruses. The larger groups, e.g., avian leukosis-sarcoma and mouse C-type, are divided into subgroups and the larger of the subgroups into types. Although these groups were originally classified on the basis of species of origin and host range, the classification is now based primarily on serological considerations, especially the relationships of virion internal proteins and of DNA polymerases, and is confirmed by nucleic acid hybridization experiments with the viral RNAs and complementary DNAs and with the viral RNAs and uninfected cell DNAs. Specificities of the relationships are seen at three levels. At the lowest level, type specific and subgroup-specific antigenic determinants or nucleic acid sequences are present only in some members of a group. At the next level, group-specific determinants or sequences are present in all members of a group and are not present in members of other groups. At the third level, intergroup-specific determinants or sequences are present in members of more than one group. Evidence for these specificities is provided by results of experiments involving genome RNA, virion DNA polymerase, internal proteins, and envelope glycoproteins. The questions are posed as to why relationships between different groups of RNA tumor viruses and those between RNA viruses and cells exist so widely in higher vertebrates and how the relationships originated. A protovirus hypothesis is offered as an explanation, stating that RNA-directed DNA polymerase activity exists in normal animals, that it plays a role in normal cellular processes like differentiation, that RNA tumor viruses evolved from this activity, and that cancer arises from variational events in the functioning of this activity. Thus, RNA tumor viruses may have repeatedly evolved from normal cellular processes that were similar in related animals because of their common ancestry. (100 references)

6015 AVIAN RNA TUMOR VIRUSES: MUTANTS, MARKERS, AND GENOTYPIC MIXING. *(E.)* Vogt, P. K. (Univ. Southern California Sch. Med., Los Angeles), J. A. Wyke, R. A. Weiss, R. R. Friis, E. Katz and M. Linial. *Collect Pap Annu Symp Fundam Cancer Res* 25:191-205, 1974.

Genetic studies on avian RNA tumor viruses are discussed with respect to conditional mutants, nonconditional mutants and genotype mixing. Conditional mutants include mutants which show a coordinate effect on transformation and virus synthesis, mutants which are temperature sensitive in virus replication but not in cellular transformation and mutants which are temperative sensitive in transformation but not in virus replication. Nonconditional mutants, derived from helper-independent avian sarcoma viruses, are either transformation-defective or replication-defective. Genetic reassortment is found in several crosses between avian sarcoma and leukosis viruses. The conditional mutants show that viral genes are required for the initiation and maintenance of cellular neoplastic transformation. However, the spontaneous appearance of transformation-defective particles which are still able to produce infectious progeny indicates that at least some of the genes needed in transformation are nonessential for virus reproduction. It is suggested that some RNA tumor viruses might be transducing agents and that transduction of genes could be important in neoplastic transformation. Genetic events leading to the spontaneous appearance of defective and reassorted viruses probably reflect the structure of the viral genome. Evidence suggests that the 70S RNA of avian RNA tumor viruses is composed of subunits of about 35S which are linked by hydrogen bonds. The replication process may at one stage produce dissociated RNA subunits which are then assembled into progeny viruses. The transformation-defective and replication-defective particles may be among the viruses that receive less than a full complement of RNA subunits. Double infections would lead to a mixing of subunits derived from different parents and could account for the high incidence of genetic combination from unmixed harvests.

The segmentation of the genome places the RNA tumor viruses in a category with the reo and influenza viruses. The spontaneous emergence of defective particles is common but not unique to viruses with a segmented genome; defectives with deletions may also occur in viruses whose total genetic information resides in a single molecule. (42 references)

6016 TUMOR VIRUS GENETICS.

(E.) Kit, S. (Baylor Coll. Med., Houston, Tex.). *Collect Pap Annu Symp Fundam Cancer Res* 25:143-157, 1974.

Studies with viral mutants can provide a direct way of relating viral gene functions to biochemical changes in infected cells. Several classes of oncogenic simian virus 40 (SV40) and polyoma virus mutants and of leukemia and sarcoma virus mutants are briefly reviewed. The SV40 and polyoma virus mutants are classified as: plaque morphology mutants, deletion mutants, adenovirus-SV40 hybrids, defective lysogens, host-dependent mutants and temperature-sensitive mutants. Representative leukemia and sarcoma virus mutants (avian and murine) are: host range variants, sarcoma virus mutants which transform chick embryo iris epithelium cells to round, nonpigmented cells or to long fusiform cells, noninfectious C-type particles, a transforming virus strain that replicates only with the assistance of a helper leukemia virus, nontrans-orming strains isolated from helper-independent sarcoma virus, mutant sarcoma virus with transforming ability but no capability to replicate, and temperature-sensitive mutants. Properties of three defective murine sarcoma strains and one avian sarcoma strain are compared. (46 references)

017 ADENOVIRUS GENES AND CANCER.

(E.) Ginsberg, H. S. (No affiliation), M. J. Ensin-er, F. E. Rubenstein and R. S. Kaufman. In: *Viru-es, Evolution and Cancer: Basic Considerations* edi-ed by E. Kurstak and K. Maramorosch (New York, cademic Press, 1974) pp. 167-181.

denoviruses produce effects which include infections ith cell death and abortive viral replication with ell transformation. Replication events are initiated y the uncoating of the viral genome and the subse-uent transcription of early species of virus-pecific RNA's. Adenoviruses have a linear, double-tranded, unsegmented DNA genome and assemble in he nuclei of cells without budding; these charac-eristics make them ideal for studying recombination. f 15 adenovirus mutants studied, 13 had only a ingle detectable defective gene function common to ll members of the specific complementation group. he wild type 12 adenovirus was found to transform rimary rat embryo fibroblasts at 39.5 C and 32 C. ransformation analysis of rat embryo cells showed hat adenovirus H5ts125 transformed cells at an ven higher frequency than the type 12 "supertrans-ormer", ts 307. Two distinct DNA minus mutants emonstrate that at least two viral gene products re required for DNA replication. At least one denovirus type (H5ts125) represents a cistron whose roduct is essential for continued DNA biosynthesis. Data suggest that all adenoviruses have the propensity to transform cells, and perhaps to be oncogenic. (51 references)

6018 STUDIES ON STRUCTURAL AND ANTIGENIC PROPERTIES OF C-TYPE VIRUSES.

(E.) Schäfer, W. (Max Planck Inst. Virus Res., Tubingen, West Germany), H. Bauer, D. P. Bolognesi, P. Fischinger, H. Frank, H. Gelderblom, J. Lange and M. V. Nermut. *Collect Pap Annu Symp Fundam Cancer Res* 25:115-140, 1974.

The electron microscope appearance, protein composition, and antigenic makeup of C-type viruses using avian (chicken) C-type virus (ATV) and murine (MuTV) C-type viruses as models are reviewed. With MuTV's, knob-like elements were observed on the viral outer membranes. The knobs were relatively loosely attached and were associated with hemagglutinating activity. The elements residing on the viral outer membrane of ATV resembled knobbed spikes and were more firmly attached than the knobs of MuTV. Recent findings indicate that the knobbed spikes of ATV may also be associated with hemagglutinating activity. The knobs and knobbed spikes represent glycoproteins possessing virus and other antigenicities, which in the case of MuTV are of the group-specific (species-specific) type. The regularly arranged subunits of the core shell seem to correspond to the ring-like structures found in the degradation products of both viruses; in MuLV these are represented by a protein with a molecular weight of approximately 150,000 daltons. Major constituents of the ATV cores are the 28,000-dalton group-specific antigen protein and a serologically unidentified 30,000-dalton protein; both constituents could be associated with the 62S viral RNA, thereby constituting the helical core filament (nucleoid). Furthermore, the viral DNA-polymerizing enzymes and the RNAse H of ATV seem to be located in the core. There is reason to suppose that the situation is similar in MuTV and that in this virus the 34,000-dalton protein(s) is contained in the core. The specific location of other proteins or protein fractions demonstrated by polyacrylamide gel electrophoresis in the virus particles is not clear. Some are certainly cellular proteins which may be associated mainly with the viral outer membrane that originates from the cell membrane. (49 references)

6019 VIRUSES WITH SEPARATELY ENCAPSIDATED COMPLEMENTARY DNA STRANDS.

(E.) Mayor, H. D. (No affiliation) and E. Kurstak. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York: Academic Press, 1974) pp. 55-78.

There are two distinct groups of small DNA viruses in which the population of virus particles contains separately encapsidated, complementary single DNA strands: the adeno-associated satellite viruses (ASV, AAV) of man and animals; and the densonucleosis virus (DNV) of the insect *Galleria mellonella* L. The similarities and differences between these viruses are reviewed. The satellite viruses and

DNV have similar biological and biophysical properties, although they show no immunological cross reactivity. In contrast to DNV, the adeno-associated satellite virus is dependent on helper action for full expression of the satellite virus genes. In the case of the latter, defective helper activities fall into three broad categories: genetically controlled complementation; environmentally controlled complementation; and host-controlled complementation. With regard to the properties of the nucleic acids within the satellite virus and DNV, DNV appears to contain a greater complement of DNA, and DNV DNA, but not satellite DNA, reassociates to double-stranded DNA when heated at high ionic strength. Acrylamide gel electrophoresis of the structural proteins of the adeno-associated satellite viruses has indicated the presence of one major and two minor components; three major and one minor protein were found in DNV. These viruses also differ in that the satellite viruses cause no known clinical diseases, while DNV is responsible for a fatal disease in the larvae of *Galleria mellonella*. Similarly, while the adeno-associated satellite viruses have an inhibitory action on adenovirus oncogenesis, DNV appears to be capable of causing transformation in mouse L cells. Future studies will define the importance of the similarities and differences between the adeno-associated viruses and DNV. (97 references)

6020 INTEGRATION OF VIRAL DNA IN TRANSFORMED CELLS. *(E.)* Blancy, D. (Salk Inst. Biol. Stud., San Diego, Calif.), R. Dulbecco, M. Haas and M. Vogt. *Collect Pap Annu Symp Fundam Cancer Res* 25:427-439, 1974.

Results of experiments to determine the number, location and physical state of viral genomes in cells transformed by oncogenic DNA viruses are summarized. The topics covered are: quantitation of viral DNA in transformed cells by DNA-RNA hybridization or by DNA reassociation kinetics, evidence for integration of the viral genome in cellular DNA, rescue of viral DNA from transformed cells, rescue by exposure to chemical or physical agents, induction of polyoma in Tsa-transformed 3T3 cells, viral genes in integration and excision, transcription of integrated viral genome, and occurrence of lytic integration. The experimental findings show that viral DNA is covalently associated with host-cell chromosomes and is probably transcribed as a large heterogeneous messenger RNA containing both viral- and cellular-specific sequences. Integration has important consequences for the virus: it allows the replication of the viral DNA together with the cellular DNA, without using viral functions; it causes the cotranscription of viral and cellular DNA, placing the viral DNA under the genetic regulatory control of the cells; and it permits the incorporation of cellular DNA into viral DNA. Integration also has consequences for the cells. Not only does it make the transformed state permanent, but it may contribute to determining transformation by interrupting the normal regulatory connections of the cellular genes. (36 references)

6021 THE BERTNER FOUNDATION MEMORIAL AWARD LECTURE — FROM PROVIRUSES TO PROTOVIRUSES: RNA-DIRECTED DNA SYNTHESIS BY RNA TUMOR VIRUSES AND CELLS. *(E.)* Temin, H. M. (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison). *Collect Pap Annu Symp Fundam Cancer Res* 25:7-38, 1974.

The histories of the DNA provirus and protovirus hypotheses are reviewed. The present status of the evidence for these hypotheses is discussed, including the recent finding of an RNA-directed DNA polymerase activity in normal chicken cells. The DNA provirus hypothesis states that replication of Rous sarcoma virus (RSV) involves formation of a DNA intermediate, which then replicates by DNA-DNA information transfer when cells replicate and by DNA-RNA transfer when progeny virus is formed. Critical evidence in favor of the hypothesis could come from confirmation of experiments indicating that an infectious DNA which contains information for RSV can be isolated from RSV-infected mammalian cells. RSV replication and transformation involve steps other than the synthesis of the DNA provirus, the intermediate for virus replication that contains the genes for virions and for transformation. Problems concerned with these other steps are discussed: specifically, the control of virus RNA synthesis, the origin of variants, the origin of virion DNA polymerase, its relationship to cell polymerases and the question of other enzymes in virions. The discovery of an RNA-directed DNA polymerase activity in normal cells, as predicted by the protovirus hypothesis, suggests some of the possible biologic effects of protoviruses (nonintegrated and integrated). These might include gene amplification, gene removal to a different location and variant sequences. Other biological consequences could be the origin of genes for neoplastic transformation, the origin of induced leukosis-like viruses and, if altered protovirus DNA were integrated in germ-line DNA, the origin of genetically altered progeny. Although laboratory RNA tumor viruses are unlikely to be implicated in human neoplasia, it is possible that RNA tumor virus-related information is related to human neoplasia and that carcinogens act on protovirus information transfers to alter normal cellular information to create the genes for neoplastic transformation. (53 references)

6022 EARLY PROTEINS OF ONCOGENIC PAPOVAVIRUSES. *(E.)* Cuzin, F. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations*, edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974) pp. 151-165, 1974.

Evidence for the use of DNA papovaviruses, simian virus 40 and polyoma as powerful tools (through immunological and genetic analysis) for studying altered growth control in malignant cells is reviewed. The existence of "pre-early" forms of virus (prior to DNA replication) leading to intranuclear and plasma membrane changes is described, the primary target still being unknown. T and U antigens located on the nuclear membrane or intranuclearly are discussed with respect to their protein composition. Efforts to identify enzyme activities associated with papovirus

nfection and involving either deletion or acquisi-
ion of enzyme are reviewed. The affinity of endo-
uclease enzyme activity for host cell (mouse) DNA
ppears to be relatively high, suggesting that the
ecognition site present on polyoma DNA might also
xist on cellular DNA. (38 references)

023 VIRUS-MEDIATED TRANSFORMATION OF MAMMALIAN CELLS. *(E.)* Rapp, F. (Milton S. Hershey
ed. Cent., Pennsylvania State Univ., Hershey). In:
evelopmental Aspects of Carcinogenesis and Immunity
dited by T. J. King (New York: Academic Press, 1974)
p. 125-136.

he virus-mediated conversion of a normal mammalian
ell into a cell with onocogenic potential is re-
iewed in general terms. In such a transformation
any properties of the cell are modified, the cell
evelops a new transformed phenotype, and many virus
inctions are expressed. Morphological changes are
nong the most readily observable modifications of
ells following transformation by viruses. Cell
irface changes are also associated with transfor-
ation. The ability of conditionally-lethal virus
itants to mediate cell transformation has been stud-
ed using temperature-sensitive mutants. These stud-
es suggest that at least two steps are involved in
ie transformation of a cell by either DNA or RNA
iruses; one step is required for the initiation of
ie transformation event and the other for the main-
enance of the transformed phenotype. Complementa-
ion tests performed with various Rous sarcoma tem-
erature-sensitive mutants also suggest that at least
vo different virus functions are required for the
kpression of the transformed phenotype. Supertrans-
ormation studies suggest that the morphological
ienotype of two different DNA viruses can be ex-
ressed in the same transformed cell. These studies
lso demonstrated that there is not just one cell-
lar site which must be modified for the expression
f cell transformation by DNA viruses; both DNA
iruses can express their own transforming potential
i the same cell. Virus particles rendered nonin-
ectious by various external agents have also been
iown to mediate cell transformation. Therefore,
ily a portion of the virus genome is required to
ediate cell transformation. There are suggestions
iat cell transformations may be more efficient if
ie ability of a virus to produce infectious progeny
id to kill the host cell is reduced. It is con-
luded that despite all the changes which have been
etected in cells transformed by viruses, there is
till no clear understanding of the actual mechanism
y which the virus modifies the expression of so many
ell properties. (41 references)

)24 MOLECULAR BIOLOGY OF TUMOUR VIRUSES. *(E.)* Edited by J. Tooze. (Imp. Cancer Res. Fund,
ondon, England). *New York, Cold Spring Harbor Lab-*
ratory, 1973, 743 pp.

overing selected work appearing since 1960, and
ntended to serve as a basic text at the graduate
evel, this book commences with origins of contemporary tumor virus research, the culture of mammalian cells, and the external surfaces of cells in culture. It continues with structure, composition, lytic cycle, transformation, and genetics of the small DNA papova viruses (polyoma and SV40). Comparable treatment is then given the larger DNA viruses (adeno and herpes) and the RNA tumor viruses (leukemia and mammary tumor). Replication, genetic transmission, and genetic analysis of RNA tumor viruses are given detailed attention at the end. An effort is made in the presentation to make the current status of tumor virus research understandable.

6025 THE EPSTEIN-BARR VIRAL GENOME AND ITS INTERACTIONS WITH HUMAN LYMPHOBLASTOID CELLS AND CHROMOSOMES. *(E.)* Pagano, J. S. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 80-115.

The relationship between the Epstein-Barr virus and human cells and tissues is reviewed. The virus and viral DNA are discussed, as are techniques of nucleic acid hybridization and interactions of the viral genome present in various tumor tissues. In particular, the viral nucleic acid of the Epstein-Barr virus has been demonstrated in nearly 100% of the tested cases of African Burkitt's lymphoma and African nasopharyngeal carcinoma. The role of the virus in the pathogenesis of the former disease is especially intriguing in that the Epstein-Barr viral DNA has not been found in the American form of Burkitt's lymphoma. The association of this virus with nasopharyngeal carcinoma is significant on two counts: this malignancy has a wider worldwide distribution than Burkitt's lymphoma; and the tumor cells are of epithelial, rather than lymphocyte origin. The association between the Epstein-Barr virus and another disease, infectious mononucleosis, coupled with the evidence that the latter is a self-limited leukemia suggests that failure of the body's immunologic surveillance mechanism following recovery from infectious mononucleosis might result in the emergence of a B lymphocyte leukemia. In light of such evidence, it becomes important to ascertain not only that the viral DNA is in the tissue but to determine the nature of the relationship between the viral genome and the cellular chromosomes. (47 references)

6026 EPIDEMIOLOGICAL EVIDENCE OF THE ROLE OF VIRUSES IN HUMAN CANCER WITH SPECIAL REFERENCE TO LYMPHOMAS. *(E.)* Davies, J. N. P. (Albany Med. Coll., N.Y.) and N. J. Vianna. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:49-58, 1974.

An epidemiologist must consider how a particular disease of unknown etiology is likely to be an infective disease. The disease must be shown to be environmentally determined; time-space clusters of disease are referred to as epidemics. Burkitt lymphoma in Ugandans was shown to be a childhood disease peaking between 5-9 yr; the few adults seen with the same clinical features were immigrants from Rwanda-Urundi. Geographic study suggested that this lymphoma might be due to a virus spread by an insect vector; this suggestion led to the discovery of that virus. Burkitt lymphoma occurs in an endemic fashion but can occur in an epidemic outbreak. In strict clinical epidemiologic terms, there is no evidence that Burkitt lymphoma is an infective disease, transmissible from person to person or animal to person. Epidemiologic evidence suggests that Hodgkin's disease is due to a transmissible agent, that involution or surgical removal of tonsils and appendix increases the liability to disease. In epidemic situations studied so far, giant follicular lymphoma, lymphocytic lymphoma and histiocytic lymphoma have been found to occur in associates of the contacts who appear to be spreading Hodgkin's disease, but no Burkitt lymphoma or leukemias have been encountered. In Hodgkin's disease, there is no defined possible causative factor, but a series of defined epidemiological features point to its infective nature and suggest how the disease may spread. (50 references)

6027 VIRUS AND BREAST CANCER. *(Fr.)* May-Levin, F. (No affiliation). *Gynecologie* 25(5):487-492, 1974.

Although the viral origin of a large number of mouse mammary tumors has been known for at least 40 yr, the idea that a virus might play a role in the development of human breast cancer has only been advanced recently. In women, as in animals, the evolution of breast cancer seems related to genetic, geographic and hormonal factors. Epidemiologic, biochemical and immunologic studies seem to point to the presence of a virus in human breast tumors. However, the presence of a virus does not necessarily mean that it is the causative factor. Evidence of type B viral particles similar to mouse mammary tumor virus (MTV) and evidence of reverse transcriptase, indicating the presence of viral RNA, were found in the milk of women with a history of breast cancer. (18 references)

6028 THE LATENT HERPES SIMPLEX VIRUS. *(E.)* Docherty, J. J. (Dep. Microbiol., Pennsylvania State Univ., University Park) and M. Chopan. *Bacteriol Rev* 38(4):337-355, 1974.

An attempt was made to organize recent reports on the latency of herpes simplex virus (HSV) based on previously proposed hypotheses. Beginning with the factors that tend to suppress herpetic infections, the virus was then followed through the various aspects of the latent state. Current methods of treatment and the potential involvement of HSV in oncogenic process were discussed. Despite the fact that experiments do not prove a viral etiology of human cancer, they do suggest that the relationship between HSV and cancer may be more than just coincidental. This in itself justifies a closer examination of the life-long and intimate association between HSV and its host during the state of latency. If the virus enters a state of latency after exposure to various chemical or physical agents, it may carry an increased ability to transform cells. The continuing presence of the virus may permit enough interaction for a carcinoma to develop. Physically- or chemically-altered HSV may increase this potential, and investigation of these possibilities is suggested. (268 references)

6029 AN OVERVIEW OF CANCER IMMUNOLOGY. *(E.)* Ritts, R. E., Jr. (Mayo Grad. Sch. Med., Univ. Minnesota, Rochester) and H. B. Neel III. *Mayo Clin Proc* 49(2):118-131, 1974.

Recent findings concerning the immunobiology of cancer are reviewed. Spontaneous, chemically-induced, and virally-induced malignant tumors in animals have been found to possess cell-surface antigens which exist neither in the normal tissues of the host nor in the tissues of animals isogenic to the host. The major immunologic defense against cancer seems to be cell-mediated, but recent evidence indicates that a humoral blocking factor enhances tumor growth. Oncogenesis also appears to involve a loss of "self" markers on the tumor cell surfaces as indicated by an unmasking of the mature differentiated surface antigens. Contrary to some results with animals, the human host's immune system shows a diminishing ability to cope with a neoplastic growth. In fact, with respect to the cellular expression of immune competency in human cancer, any defect appears to be a general one manifest to all antigens rather than a specific one related to a specific tumor antigen. At least in chronic lymphatic leukemia, the apparent functional, putative T-cell-regulated deficit is based on the absence of T cells and the overproliferation of B cells. Although there is little data regarding the host's immune status in solid-tissue neoplasia, elevated complement activity has been found in patients with untreated malignancies of the bowel. The diminution or absence of delayed-type hypersensitivity or cellular immunity has been frequently reported to tuberculin, skin allografts, cancer-cell-line implants, keyhole limpet hemocyanin, and substituted nitrobenzenes, and there may be an additional aberration in the expression of inflammation. The failure to manifest cellular immunity in cancer may be due to a deficit and/or a malfunction of the thymus-regulated lymphocytes. As to why tumors are able to arise and proliferate despite the host's immunologic defense mechanisms, it is possible that the defense fails to recognize the tumor antigens, which are weak and little different from self. The proliferating tumor is then able to outpace normal defense mechanisms and spread beyond them. (204 references)

6030 CANCER AND IMMUNITY. *(Fr.)* Cerottini, J. C. (Swiss Inst. Exp. Res. Cancer, Lausanne, Switzerland) and J. P. Mach. *Rev Med Suisse Romande* 94(9):675-684, 1974.

The study of the relationship between cancer and the body's immune system has addressed itself to three questions. Do tumor cells differ, in an antigenic sense, from the normal cells from which they derive?

re the differences sufficient to trigger immune esponse in the host organism? In what way does mmune response play a role in controlling tumor rowth? Recent studies have shown that the structure f human neoplastic cells differs quantitatively from hat of normal tissue. In some cases, the change in tructure seems capable of triggering immune response n cancer patients, but it still remains to be emonstrated that this immune response plays a role n the destruction or inhibition of tumor growth. t is hoped that further knowledge regarding the ature of tumor antigens, the mechanism of antitumor mmunity and the means of potentiating this immunity ill eventually lead to rational immunotherapy. (No eferences)

031 HOST IMMUNE RESPONSE TO VIRUS-INDUCED TUMORS: SOME RECENT CONCEPTS. *(E.)* Aoki, T. Natl. Cancer Inst., Natl. Inst. Health, Bethesda, d.), L. Chieco-Bianche, E. J. Plata, F. Sendo, V. W. ollis, Jr. and T. Kudo. *Prog Exp Tumor Res* 19:23-26, 974.

ome recent aspects of animal host responses to tuors have been selected and reviewed, namely: the ack of complete immune tolerance, tumor-associated ransplantation antigens in F_1-hybrid mice, antigenic odulation, naturally occurring humoral antigens and ntibodies and the relationship between humoral and ell-mediated immune responses. Exceptions to the onventional view of immune tolerance are cited and ne bases for rejection of spontaneous and translanted tumors are discussed. Rejection of tumor of nbred strains by F_1-hybrids may throw light on mechnisms of tumor rejection; similarly the phenotypic nange from cell surface antigen$^+$ to antigen$^-$ by cerain leukemias after transplantation may partly exlain progressive tumor growth. The balances between oluble antigens, their respective antibodies and esultant complexes are discussed with respect to ost immune-response and various reasons for the failre of demonstrable cell-mediated immunity to prevent mor growth are presented. The problem of host imne-response to tumors is complex and unresolved but rther work using animal models for human cancer may rove useful in the development of clinical immunoerapy. (65 references)

)32 CONTROL OF PROLIFERATION IN THE IMMUNE SYSTEM. *(E.)* Fahey, J. L. (Dep. Microiol. Immunol., Univ. California, Los Angeles). *ld Spring Harbor Conf Cell Proliferation* 1:379-2, 1974.

mponents of the immune response and processes miting the immune response are reviewed. Relaionships between the following components of the ntibody response are summarized: antigen/immunon, immune competent clones, responding clones, elper systems, proliferation and differentiation, nd recruitment. Processes limiting the immune esponse are feedback control by antibody binding antigen, exhaustion of clonal proliferation apacity, maturation, diversion, clonal dominance, Gompertzian kinetics of proliferation, suppressor T cells, suppressor factors, and intracellular control of immunoglobulin production. Studies with human lymphoid cells in continuous culture have indicated two patterns of immunoglobulin synthesis: synthesis concentrated in the late G_1 or early S phases of the cell cycle and more or less continuous synthesis with waves of increase in late G_1 and G_2 with a reduction at mitosis. These findings emphasize that immunoglobulin synthesis does not have a uniform relationship to the division cycle in all cells. Synchronous doublings and waves of antibody and DNA synthesis before the first mitosis are features of both lymphoid cells in tissue culture and of antigen-sensitive cells *in vivo*. These similarities indicate the possibility of common pathways of immune cell stimulation and of growth limitation. (41 references)

6033 IMMUNOSUPPRESSION AND ONCOGENESIS. *(E.)* Cerilli, J. (Dep. Surg., Ohio State Univ., Columbus) and D. Hattan. *Am J Clin Pathol* 2(2):218-223, 1974.

The potential oncogenic effects of manipulating and depressing the immune system are reviewed. The increased incidence of malignancies in immunosuppressed animals has been demonstrated with a wide variety of virus- and carcinogen-induced tumors. Immunosuppression increases mortality, the rate of tumor growth, the percentage of tumor "takes" and the incidence of metastases in animals receiving transplanted tumors. These effects are most pronounced when immunosuppression is initiated prior to tumor inoculation. In man, immunosuppression favors the growth and, in some cases, increases the incidence of metastases following tumor transplantation. This phenomenom has been observed following the inadvertant transplantation of tumor along with renal homografts. Immunosuppression also increases the incidence of spontaneous tumors in mice, and immunosuppressed non-transplant human patients manifest an affinity for developing spontaneous neoplasms. With the exception of antilymphocyte sera, immunosuppression imposed on an already established tumor has little effect. Several theories have been advanced to explain the increased incidence of *de novo* neoplasms in immunosuppressed hosts. The immunologic surveillance theory suggests that tumor cells are constantly developing, but that they evoke an immune response which normally leads to their own rejection. If these immunologic response mechanisms are depressed, a *de novo* cancer is more likely to develop. A second theory suggests that chromosome aberrations resulting directly or indirectly in neoplasms may occur in immunosuppressed hosts. According to another theory, the decreased immunologic responsiveness of the immunosuppressed host could facilitate the growth of oncogenic viruses, and a fourth theory holds that malignancies in transplant patients may be due to antigenic stimulation caused by the graft. More evidence is needed to determine which, if any, of these theories is correct although sufficient evidence now exists to warrant close observation for the development of malignancy in patients on immunosuppressive therapy. (28 references)

6034 OCCURRENCE OF CANCER IN IMMUNE DEFICIENCIES.

(E.) Penn, I. (Univ. Colorado Sch. Med., Denver). *Cancer* 34(3):858-866, 1974.

Several clinical types of immunodeficiency in which there is either unequivocal or else circumstantial evidence of an increased incidence of malignancy are analyzed and described. In primary immunodeficiency states, the frequency of malignancy is roughly 10,000 times that in the general age-matched population, while immunosuppressed organ homograft recipients have a 5-6% risk of developing *de novo* cancers. In addition, malignant cells may be inadvertantly transplanted from donors with cancer and may grow in the transplanted organ, invade surrounding structures, and produce widespread metastases. Cancers may also develop in nontransplant patients treated with immunosuppressive drugs, and circumstantial evidence suggests that cancer chemotherapy, while allowing control over one cancer, may permit or induce the growth of a second neoplasm. These findings have important implications for the management of patients with malignancies, since other forms of cancer treatment such as radical surgical procedures and radiotherapy may also impair the host's resistance to cancer. Reappraisal of the current methods of cancer therapy is recommended to determine their effects on the host's resistance to his neoplasm. (76 references)

6035 BLOCKING FACTORS IN TUMOR IMMUNITY AND ALLOGRAFT TOLERANCE.

(E.) Hellström, K. E. (Univ. Washington Med. Sch., Seattle), I. Hellström, P. W. Wright and I. D. Bernstein. In: *Immunological Tolerance: Mechanisms and Potential Therapeutic Applications* edited by D. H. Katz and B. Benacerraf. New York, Academic Press, 1974, pp. 565-585.

The molecular nature of serum blocking factors and their role in facilitating tumor growth and in maintaining allograft tolerance *in vivo* are reviewed. In most systems studied, lymphocytes from animals and human patients with growing tumors have been found to be capable of killing cultivated cells from the respective neoplasms. Serum from animals and humans with growing tumors can inhibit this cytotoxic effect. The blocking effect is specific in that sera from individuals with tumors of "other" antigenicity do not block, and blocking activity is generally absent from sera taken from individuals which are free of tumor or whose tumors have regressed or been removed. The blocking factors appear to be of two kinds: antigen-antibody complexes and free antigens. The major point of attack seems to be the effector cell and there is evidence that both T and B cell reactivity can be blocked. Studies on both animals and human tumor patients have established a positive correlation between serum blocking activity *in vitro* and the presence of tumor growth *in vivo*. The ability of tumor cells to release antigens which block lymphocyte-mediated cytotoxicity and/or form complexes which do so may provide an important escape mechanism for neoplastic cells from immunological control. Some tumor antigens, particularly the embryonic antigens, detected *in vitro* may appear to be weak or nonexistent *in vivo* just because they are particularly good blockers. In addition, various studies have shown that cytotoxic lymphocytes and blocking serum factors are present in operationally defined allograft tolerance; the blocking factors in tolerant sera are antigen-antibody complexes, the antibody part being IgG immunoglobulin. The role of blocking factors in maintaining allograft tolerance is unclear at this time. (40 references)

6036 ALTERATIONS IN MEMBRANE STRUCTURE AND FUNCTION ASSOCIATED WITH NEOPLASTIC TRANSFORMATION *IN VITRO.*

(E.) Patterson, Jr., M. K. (Biomed. Div., Samuel Roberts Noble Found., Inc., Ardmore, Okla.). *J Natl Cancer Inst* 53(5):1493-1498, 1974.

The degree to which alterations in membrane structure and function associated with *in vitro* neoplastic transformation reflect universal changes amenable to rapid assay procedures is reviewed. Factors which have been reported to effect changes in the plasma membrane unassociated with transformation include cell density, variations in the constituents of growth media, washing of cells, cell harvest procedures, and the presence of contaminants. In relating lectin binding, agglutination, and tumorigenicity in transformed cells, the binding of lectins was found to be a necessary but not sufficient condition for agglutination. Antigenic alterations of cells after transformation are difficult to classify due to the different immunologic responses of cells transformed by different systems. Concentration of L-fucose is consistently lower in transformed cells and membrane glycoprotein is altered. Altered transport mechanisms are found in the cell surface membranes of transformed cells. Although alterations in membrane glycoprotein and nutrient transport may be universal changes associated with transformation, the usefulness of specific changes in structure and function as indicators of neoplastic transformation *in vitro* await further study. More data on chemically and spontaneously transformed cell lines are needed and stringent control over culture conditions is required. The areas under investigation in systems of transformation *in vitro* may provide means for the detection and even control of the malignant state. For example, immunologic reactions against tumor cell surface antigens may help in immunotherapy, and carbohydrate expressions of the cell surface may provide selective targets for chemotherapy. (81 references)

6037 MEMBRANE ANTIGENS OF THE COLONIC TUMORS.

(E.) Burtin, P. (Inst. Rech. Sci. Cancer, Villejuif, France). *Cancer (Suppl.)* 34(3):829-834, 1974.

Current knowledge concerning the membrane-associated antigens which have been identified in gastrointestinal adenocarcinomas in man is reviewed. Immunochemical studies have demonstrated the existence of the carcinoembryonic antigen (CEA), a nonspecific cross-reacting antigen (NCA), and a membrane-associated tissular autoantigen (MTA). CEA is a heterogeneous glycoprotein with a molecular weight of about 200,000 daltons. It has consistently been found in adenorcinomas of the colon and rectum and in their me- stases. It has also been found in the mucosa of the tal colon, in digestive tumors maintained in organ lture, in human colonic tumors serially propagated hamsters, and in some permanent lines of colonic mor cells. CEA appears to be associated with the ical pole membrane of most cells, from which po- tion it is probably rapidly excreted. NCA is a ycoprotein with a molecular weight of about 50,000 ltons which cross reacts with CEA and has a common tigenic structure. NCA has been found in many rmal and cancerous tissues and in the plasma. It situated at the excretory pole of the glandular lls at the surface of invasive cells and in intra- andular deposits. MTA is a glycoprotein with a lecular weight of about 30,000-40,000 daltons; it s no antigen relationship with CEA or NCA. This tigen has been detected in many normal and cancerous ssues, is associated with the cell membrane, and ows an autoantigenicity which is probably related its membrane localization. Autoimmunity studies ve demonstrated the presence of antigens, probably cated on the cell membranes, which are involved in ecific cellular immunity in patients with carcinoma the colon. These antigens are unlikely to be re- ted to those characterized by immunochemical me- ods. (39 references)

38 α-FETOPROTEIN: EXPRESSION IN HUMAN DISEASE AND IN RAT EXPERIMENTAL MODELS. *(E.)* Wep- c, H. T. (Univ. California San Diego Med. Sch., Jolla) and S. Sell. *Prog Exp Tumor Res* 19:297-324, 74.

ecent observations on the relationship of α-feto- otein to human malignancy and other disease states e reviewed and compared to a rat-model system. man α-fetoprotein has been isolated by immunoab- rption; immunization of an animal with these pre- rations results in an antiserum which is specific r α-fetoprotein, without absorption. The α-feto- otein of normal serum ranges from 1.5-16.5 ng/ml d is the same as that detected at higher concen- ations in pathological states. When α-fetoprotein- sitive hepatic tumors are detected, it is often ar the end of the disease course; approximately % of sera from patients with primary hepatomas ntained α-fetoprotein. Correlation of serum α- toprotein with malignancy appears more frequently in unger hepatocellular carcinoma patients than older tients and at higher serum levels in men with hepatic mors than women. α-Fetoprotein is one of two oteins found in rat amniotic fluid and in newborn t serum; fast growing, poorly differentiated ansplantable rat hepatic tumors have higher α- toprotein serum levels than more slowly growing mors. In patients undergoing complete removal a hepatoma, a marked fall in serum α-fetoprotein vels is noted with half-life of 4-7 days. Studies patients undergoing liver transplantation showed at α-fetoprotein levels were apparently unaffec- d by immunosuppressive therapy, chronic transplant jection, recurrent infections, or hepatic crosis during rejection episodes. Presence of tastatic Morris hepatoma 7777 tumor in rats could predicted by rising α-fetoprotein serum levels up to 30 days prior to detection by physical examination. Little change was noted in α-fetoprotein serum concentrations in patients undergoing chemotherapy for hepatoma. Production of α-fetoprotein has been observed in germinal cell malignancies and other non-hepatic human tumors. Determination of circulating α-fetoprotein may be used to differentiate between choriocarcinoma or hydatidiform mole and normal pregnancy. (131 references)

6039 THE ASSOCIATION OF THE HL-A ANTIGENS WITH DISEASES. *(E.)* Dausset, J. (Lab. Immunol. Hematol., Univ. Paris, France), L. Degos and J. Hobs. *Clin Immunol Immunopathol* 3(1):127-149, 1974.

The possible mechanisms of association between the HL-A antigens and various neoplastic and non-neoplastic diseases are reviewed. Experimental data indicate some correlation between a susceptibility to oncogenic viruses and the immune response and the H-2 system in mice; the H-2 system corresponds to the HL-A system in man. However, in man there is no clear association between any of the HL-A antigens and acute lymphocytic or other types of leukemia. There is a close association between HL-A8 and gluten enteropathy, dermatitis herpetiform and systematic lupus erythematosus, while ankylosing spondylitis, psoriatica arthritis, Reiter's disease, and Yersinia arthritis are associated with W27 and psoriasis and pemphigues are associated with HL-A13. These diseases, as well as several others which are associated with various HL-A antigens must be distinguished from those, such as ragweed pollen allergy, in which there is an apparent link between the HL-A system and an abnormal gene. With regard to the diseases which are associated with a given HL-A antigen, it is possible that the HL-A products are themselves responsible for the association with disease; given the well-established presence of Ir genes in mice and guinea pigs, it is also possible that similar genes exist in man. The fact that Ir genes are probably present in the HL-A complex permits the prediction that heterozygotes in this region will be able to develop a larger spectrum of antibodies and will accordingly enjoy a biological advantage; there is some evidence that homozygotes are more susceptible to disease and especially to cancer. The HL-A complex may well find its ultimate mission in its relationship with selection, as well as in transplantation. (164 references)

6040 MORPHOLOGY AND HISTOLOGICAL TYPES OF HODGKIN'S DISEASE. *(It.)* Lanza, G. (Inst. Anat., Univ. Ferrara, Italy) and I. Nenci. *Recent Prog Med (Roma)* 55(4):288-309, 1973.

Problems involved in achieving classification of Hodgkin's disease that would minimize diagnostic ambiguities are reviewed. After a brief survey of the history of the histopathological classification, the validity of the Sternberg cell in diagnosis is affirmed, variations being explained as functions both of the immune reaction and of the effect on diverse types of cells. A detailed examination of the international classification into four histologic types is given. The view of their interrelation as (1) variants of a basic disease process influenced by factors discernible through epidemiological methods, (2) having no diagnostically conclusive association with anatomical distribution, yet (3) closely correlated with the four clinical stages and (4) delineating progressively malignant illnesses with negative prognoses is affirmed. The nosographic, morphological and biological unity of this poliphasic variable malignancy is reasserted on the basis of an immunomorphological model. (88 references)

6041 CROHN'S DISEASE AND MALIGNANT TRANSFORMATION. *(Fr.)* Chevrel, B. (St. Antoine Hosp., Paris, France). *Med Chir Dig* 3(6):431-436, 1974.

The coexistence of Crohn's disease and intestinal cancer seems to occur too frequently to be mere coincidence, especially since this particular cancer is specific as to age, site and histological distribution. The relationship between Crohn's disease of the ileum and cancer seems too infrequent to call for preventive surgery. However, the frequency with which cancer appears on by-pass segments makes resection preferable if surgery is undertaken. In Crohn's disease of the colon, radical surgery is advised since malignant degeneration occurs with more frequency. The possibility of malignant change must be considered in all cases, especially if associated with an alarming clinical picture. Surgery should be weighed carefully knowing that negative histological results of a portion of exeresis tissue does not eliminate the possibility of a malignant tumor located nearby or on another intestinal segment. Although of unproven value in mass studies, the only detection test available at present is repeated analysis of carcinoembryonic antigens (CEA). The need for a diagnostic test is imperative since prognosis for these cancers in young people is extremely poor. (55 references)

6042 CELL SURFACE CHANGES AND NEOPLASTIC PHENOTYPE(S). *(E.)* Emmelot, P. (Netherlands Cancer Inst., Amsterdam). *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:3-9, 1974.

Biochemical changes observed in neoplastic cell surfaces are reviewed to determine whether uniform changes exist in transformed cells *in vitro,* and whether such changes may also occur in tumor cells *in vivo.* Increased cholesterol content was observed in five types of hepatoma as compared with normal liver plasma membranes. In the anaplastic, rapidly-growing rat hepatoma, the increase in cholesterol was accompanied by an increase in saturation in fatty acids; in two slow-growing and well-differentiated mouse hepatomas there was hardly any increase in saturation in fatty acids. In the surface of all transformed and malignant cells examined, there is an increase of sialic acid in fucose-containing glycoproteins. This has been established for seven strains of transformed and malignant cells of three morphologies and seems to be the only universal difference between cell surface properties of normal and neoplastic cells. Temporal changes in glycolipids, glycoprotein and agglutinin receptors occur in the normal cell surface as a function of the growth cycle. Tumor cell surface acts like a static entity, blocked in the mitotic surface expression. (68 references)

6043 GENETICS AND CANCER. *(Fr.)* Rouesse, J. (No affiliation). *Gynecologie* 25(5):485-486, 1974.

The role of genetics in cancer, although long suspected, is still poorly understood. Conclusions drawn from experimental data vary greatly and sometimes point to the possible role of genetic factors in carcinogenesis. Clinical and cytogenetic results in human pathology are even less conclusive. Human studies regarding the relationship between particular genes and the occurrence of particular types of cancer seem promising. (No references)

6044 THE PHILADELPHIA CHROMOSOME AND CHRONIC MYELOCYTIC LEUKEMIA (CML) -- STILL A COMPLEX RELATIONSHIP? *(E.)* Gahrton, G. (No affiliation), J. Lindsten and L. Zech. *Acta Med Scand* 196(5):353-354, 1974.

A brief editorial review of recent literature concerning the Philadelphia (Ph^1) chromosome and CML is briefly reviewed. The incidence of the Ph^1 chromosome in CML patients is about 85%, and the formation of the Ph^1 chromosome is believed to be the result of a translocation between chromosomes 9 and 22. Unresolved problems concerning the Ph^1 chromosome include how the translocation is induced, etiological agents and whether it is the result or the cause of CML. (18 references)

6045 GASTRIC CANCER: AN EPIDEMIOLOGICAL AND STATISTICAL STUDY. *(Fr.)* Hammer, B. (Cantonal Hosp., St. Gall, Switzerland). *Cah Med* 15(10):655-661, 1974.

In the past ten yr, there has been a world-wide decrease in stomach cancer mortality, yet improvement in therapeutic results has been minimal; lower morbidity might account for the lower mortality. A review of the literature indicates that stomach cancer mortality seems to vary according to geographical location, with the highest mortality rate observed in Chile (7.1 men and 4.5 women/10,000) and the lowest rate found in the white U.S. population (1.4 men and 0.58 women/10,000). Higher incidence of stomach cancer is found in lower income groups, in eastern Europe (as opposed to western Europe), in American blacks and in type A blood groups. Stomach cancer occurs mostly in the 60-70 yr age group. Poor prog-

sis is due to the fact that this type of cancer
es not present frankly recognizable symptoms for
ıths or even years. The combination of radiological
amination, gastroscopy, gastrocamera endophotog-
phy and cytologic examination provides the best
agnostic results (over 90% detection). At present,
ere is no valid alternative to surgery. The five-
post-surgical survival rate has remained more or
ss constant, at 10-20%, world-wide. Early detec-
on is still the key to improvement of the survival
te. Due to lack of funds for mass screening tests
d the absence of simple diagnostic procedures, early
tection of stomach cancer is not foreseen in Europe
the near future. (34 references)

46 THE METABOLIC ASPECTS OF NUTRITIONAL SAFETY. METABOLIC TOXICOLOGY. *(Fr.)*

emolieres, J. (Bichat Hosp., Paris, France). *Ann*
tr Aliment 28(4):199-206, 1974.

rious aspects of food safety are discussed in
rms of man, his environment, foreign substances
d what represents toxicity. Metabolic toxicology
defined as a science dealing with physiological
d biochemical changes occurring in foreign sub-
ances prior to the appearance of cell lesions.
ggested biochemical studies required to investi-
te safe use of foreign substances are as follows:
identification and purity control of additives,
determination of changes in both additive and
od caused by their interaction, 3) *in vitro* and
vivo studies of changes in the digestive tract,
chemical changes affecting the additive during
sorption, metabolic processes and distribution
roughout the organism, 5) measurement of biologi-
l half-life of metabolites and their concentra-
on in the blood, and 6) use of animals whose
tabolism closely resembles that of man. In con-
usion, man must adapt more closely to the condi-
ons of his environment. (1 reference)

47 CONTROL OF CELLULAR PROLIFERATION IN MAMMALIAN CELLS. *(E.)*

Baserga, R. (Temple
iv. Sch. Med., Philadelphia, Pa.). *Bibl Haematol*
9):978-983, 1974.

theory for the initiation of cell division is pre-
nted based on the observation of increased RNA
nthesis prior to onset of DNA replication. It is
oposed that the cellular genome is activated by
idic nuclear proteins (the product of the RNA syn-
esis "burst"), and the triggering stimuli for cell
vision act primarily upon certain RNA templates
at synthesize specific acidic nuclear proteins,
ich then reverse histone suppression of DNA-depen-
nt RNA synthesis. A cause and effect relationship
tween observation of an increase in RNA synthesis
mediately preceding DNA replication remains to be
oved. (16 references)

)48 CYCLIC AMP AND THE TRANSFORMATION OF FIBROBLASTS. *(E.)*

Pastan, I. (Natl. Cancer
ıst., Bethesda, Md.) and G. S. Johnson. *Adv Cancer*
s 19:303-329, 1974.

The relationship between cyclic AMP and neoplastic transformation of various species of fibroblasts is presented. Reasons for considering a relationship between cellular cyclic AMP levels and cancer are given and the effects of cyclic AMP, its membrane-diffusible derivatives or inhibitors of cyclic AMP diesterase (theophylline, caffeine) on normal and transformed fibroblast morphology, adhesion, motion, membrane transport and growth kinetics are described. Chemical structure specificity and effects on special processes such as growth of transformed cells in agar, collagen and mucopolysaccharide synthesis, *in vivo* tumor growth, viral replication, carcinogenesis and membrane composition are considered. Methods for assay of cyclic AMP levels, factors affecting the levels and studies on the cyclic AMP enzymes (adenylate cyclase and cyclic AMP phosphodiesterase) in normal and transformed fibroblasts are reviewed. Many of the factors that cause cancer also result in lowered cellular cyclic AMP levels; adenylate cyclase seems to be the target in viral transformation. (84 references)

6049 TUMOR-ASSOCIATED FIBRINOLYSIS. *(E.)*

Reich, E. (Rockefeller Univ., New York, N.Y.). *Cold Spring Harbor Conf Cell Proliferation* 1:351-355, 1974.

This review deals with enzymatic differences between normal and malignant cells obtained in recent experiments in which virus-transformed fibroblasts grown on thin fibrin films showed rapid fibrinolysis and normal controls did not. Fibrinolysis is associated with transformation of avian and mammalian cells in culture by either DNA or RNA viruses; it is observed in primary cultures of chemically induced mammary carcinomas, hepatomas, and skin cancers; and is found in a variety of human and animal tumor cell lines. Optimal expression of fibrinolysis depends on the nature of the serum supplement, and a characteristic spectrum of activating and non-activating sera is obtained for different transformed cultures; serum specificity is determined by the cell type and not by the transforming agent. The mechanism by which neoplastic cells initiate fibrinolysis depends on a serum factor, plasminogen, and on a cell factor, serine protease. The latter is a plasminogen activator. Characteristic changes in cell morphology, colony formation in semi-solid media, and enhanced cell motility all require the presence of an intact fibrinolytic system. Recent studies also show that transformed cells produce receptors for plasmin and that normal cells develop morphological changes characteristic of transformation when cocultivated with transformed cells under conditions permitting activation of the fibrinolytic system. Some of the serum effects on these processes may be attributable to differences in levels of enzyme activity. Disorders in bleeding, clotting, and fibrinogen metabolism indicate that the fibrinolytic system is operating at pathological levels in human malignant disease. (9 references)

6050 MINIREVIEW: POSTREPLICATION REPAIR OF DNA IN MAMMALIAN CELLS. *(E.)*

Lehmann, A. R. (M.R.C. Cell Mutat. Unit, Univ. Sussex, Falmer, England). *Life Sci* 15(12):2005-2016, 1974.

The postreplication repair of DNA in mammalian cells is discussed, principally with regard to damage caused by ultraviolet (UV) irradiation. In strains of *Escherichia coli* unable to excise pyrimidine dimers, the newly-synthesized DNA in UV-irradiated cells was initially of lower molecular weight than in unirradiated cells. The low molecular weight DNA gradually increased on subsequent incubation, eventually reaching the same size as that of DNA from unirradiated controls. Evidence indicates that the DNA replicated in the irradiated cells contained single-stranded regions resulting from gaps opposite the dimers. The gap-filling was achieved by a process involving sister-strand recombination exchanges or large amounts of DNA, the dimers becoming equally distributed between the parental and daughter strands. A similar process seems to occur in mammalian cells, which also synthesize low molecular weight DNA following UV irradiation. There is no evidence supporting the bacterial model of gap-filling by recombinational exchanges in mammalian cells. Gap-filling is inhibited by hydroxyurea, cytosine arabinoside, excess thymidine, actinomycin D, and caffeine, but not by cycloheximide. In some, but not all mammalian cells, the ability to synthesize DNA of normal molecular weight is recovered within 6-8 hr following irradiation. Xeroderma pigmentosum (XP) cells show a caffeine-sensitive deficiency in postreplication repair following UV-irradiation, although they demonstrate normal excision repair. Deficiencies in postreplication repair and various defects in excision repair at the cellular level seem to give rise to similar clinical symptoms (skin cancer) in man. A caffeine-sensitive S-phase specific postreplicative repair process has also been observed in cells treated with alkylating agents and seems to resemble the dimer/gap mechanism for postreplication repair in UV-irradiated cells. A possible link between postreplication repair and mutation induction is suggested by findings that caffeine affects both processes. (68 references)

6051 CARCINOGENESIS AND DNA REPAIR. *(E.)* Van Lancker, J. L. (Univ. California Cent., Health Sci., Los Angeles). *Biochem Dis* 4(Part A):427-440, 1974.

The relationship between carcinogenesis and DNA repair mechanisms is reviewed. Since UV light, X-irradiation, and carcinogens modify cellular DNA chemically, it is logical to postulate that DNA is the primary target for an injury that ultimately leads to a stepwise distortion of gene expression and release of cells from normal controls over cellular proliferation, maturation, and interreactions. In intact animals, repair of X-irradiated DNA appears to occur in two stages: one rapidly following the administration of irradiation, and the other developing weeks after exposure. It also appears that damage to repressed DNA is not readily repaired; thus damage inflicted on DNA in the repressed state persists, while such damage is eliminated from DNA in the derepressed state as a result of rapid repair. Additional data suggest that differences in DNA repair account for the observed higher sensitivity of heterochromatin to chromosomal alterations. If cancer does, however, result from point mutation, deletion, or intercalation, are these alterations due to the DNA alteration itself or to the action of the repair enzymes? It is conceivable that attempts to repair the damaged DNA might cause irreparable breaks or base mispairing which lead to cell transformation. (63 references)

6052 MINIREVIEW. AGENTS WHICH INHIBIT REVERSE TRANSCRIPTASES. *(E.)* Smith, R. G. (Natl. Cancer Inst., Bethesda, Md.) and R. C. Gallo. *Life Sci* 15(10):1711-1730, 1974.

A rationale for the development of inhibitors of the DNA polymerases of RNA tumor viruses (reverse transcriptases) is outlined, and a classification of these inhibitors based on the biochemical properties of the enzymes and the mechanism of action of the inhibitors is presented. There is accumulating evidence that RNA tumor viruses are involved in at least some forms of human cancer, such as leukemia. In animals cells transformed by RNA tumor viruses, reverse transcriptase is required only to initiate, but not to maintain, the transformed state. By extension, therefore, the rationale for the development of inhibitors of viral DNA polymerases for possible clinical use is limited to disease prophylaxis and possibly for remission maintenance. The evidence favoring this concept complements the demonstration of components of RNA tumor viruses in human leukemic cells and supports the notion that inhibitors of reverse transcriptase may be useful in halting the spread of oncogenic information within as well as possibly between humans. The ideal inhibitor is defined as a compound that is: 1) highly selective in attacking the biologically important reaction of reverse transcriptase; 2) potent enough so that effective concentrations can be easily achieved *in vivo;* 3) able to penetrate cells and 4) easy to formulate for administration to animals or man. Agents which inhibit reverse transcriptase are grouped according to the following classification scheme: 1) enzyme-binding agents; 2) substrate analogues; 3) template-primer analogues; 4) template binding agents; 5) divalent cation-binding agents; and 6) agents of unknown mechanism of action. Specific inhibitors from each group are discussed to illustrate important principles. The authors suggest that a deeper understanding of the structure and function of the reverse transcriptases may lead to the rational design of inhibitors not only of biologic, but also of clinical, usefulness. (79 references)

6053 RNA-DIRECTED DNA POLYMERASE ACTIVITY IN UNINFECTED CELLS. *(E.)* Temin, H. M. (McArdle Lab., Univ. Wisconsin, Madison) and C. Y. Kang. In: *Developmental Aspects of Carcinogenesis and Immunity* edited by T. J. King (New York: Academic Press, Inc., 1974) pp. 137-144.

Evidence in favor of the existence of RNA-directed DNA polymerase activity in normal cells and its possible role in normal differentiation are discussed.

logenous RNA-directed DNA polymerase activity bio-
emically similar to that found in virions of
odeoxyviruses has been isolated from uninfected
icken embryos and cells. The chicken endogenous
A-directed DNA polymerase activity is not directly
lated to either of the avian ribodeoxyviruses (avian
ukosis-sarcoma viruses or the reticuloendotheliosis
ruses); it has an RNA template and a DNA polymerase
fferent from that in rnadnaviruses. The DNA poly-
rase of the cellular endogenous RNA-directed DNA
lymerase activity is related to the 3-4S DNA poly-
rase of cells. There is some evidence for tissue
d phase specificity in the amount of this activity,
t its role in differentiation remains hypothetical.
6 references)

54 ELECTRONIC BIOLOGY AND ITS RELATION TO CANCER. *(E.)* Szent Gyorgyi, A. (Mar.
ol. Lab., Woods Hole, Mass.). *Life Sci* 15(5):863-
5, 1974.

fundamental role is proposed for highly reactive
ee radical carbonyl groups in biological systems
d malignant growth is suggested to be primarily the
sult of disturbance in carbonyl group reactivity.
ter-insoluble proteins are considered as the semi-
nducting transducers under aerobic conditions for
is process, and the dicarbonyl compound, methyl
yoxal mediated via glyoxylase, is considered as
ssessing the necessary physical characteristics for
free radical system for control of energy transfer
thin a cell. Electron spin resonance signals are
ted as evidence for this charge transfer mechanism
biological systems. With respect to cancer, the
eory is proposed that during continuous cell di-
sion this free radical energy control mechanism
itches off and proliferating tissues, including
ncer, are characterized by low electron spin
sonance signals. Better understanding of the
ee radical dicarbonyl-mediated energy transfer
ntrol mechanism may prove valuable in finding
e cause and means of preventing cancer. (18
ferences)

55 METABOLIC ACTIVATION OF DIMETHYLNITROSAMINE AND DIETHYLNITROSAMINE TO MUTAGENS.
.) Malling, H. V. (Natl. Inst. Environ. Health
i., Research Triangle Park, N.C.) and C. N. Frantz.
tat Res 25(2):179-186, 1974. (18 references)

56 THE GORDIAN KNOT OF TUMOUR IMMUNOLOGY. *(E.)* Amlot, P. (Guy's Hosp., London, Eng-
nd). *Guys Hosp Rep* 123(1):43-51, 1974. (20 refer-
ces)

57 THE EXPERIMENTAL EXPLORATION OF HEALTH DAMAGING FACTORS IN CIGARETTE SMOKE. *(E.)*
uchtenberger, C. (Swiss Inst. Exp. Cancer Res.,
usanne, Switzerland) and R. Leuchtenberger. *Soz
aeventivmed* 19(1):41-45, 1974. (24 references)

6058 THE INTERACTION OF CIGARETTE SMOKE WITH THIOL GROUPS, A MODEL STUDY. *(E.)* Stauffer, H. P. (Burgdorf Coll. Technol., Dep. Chem., Switzerland). *Soz Praeventivmed* 19(1):55-58, 1974. (33 references)

6059 PRIMARY MELANOMA OF THE LEPTOMENINGES: A REVIEW. *(E.)* Savitz, M. H. (Mount Sinai Sch. Med., City U. New York, N.Y.) and P. J. Anderson. *Mt Sinai J Med NY* 41(6):774-791, 1974. (154 references)

6060 ON THE INTERRELATION BETWEEN BRONCHIAL CARCINOMA AND NEOPLASM OF THE URINARY TRACT. *(E.)* Anonymous. *Acta Pathol Microbiol Scand [A]* (Suppl. 247):35-66, 1974. (71 references)

6061 BERYLLIUM: AFTER FORTY YEARS, STILL A MYSTERY. *(E.)* Anonymous. *Environment* 16(3):35-36, 1974. (No references)

6062 RENAL NEOPLASMS IN CHILDREN. *(E.)* Kandzari, S. J. (West Virginia U. Med. Ctr., Morgantown), S. L. Hill and D. F. Milam. *W Va Med J* 70(10):259-261, 1974. (No references)

6063 α-FETOPROTEIN AS A MARKER OF EMBRYO-SPECIFIC DIFFERENTIATIONS IN NORMAL AND TUMOR TISSUES. *(E.)* Abelev, G. I. (N. F. Gamaleya Inst. Epidemiol. Microbiol., Moscow, USSR). *Transplant Rev* 20:3-37, 1974. (118 references)

6064 ANIMAL MODELS FOR BRAIN TUMOR RESEARCH. *(E.)* Swenberg, J. A. (Pathol. Toxicol. Res. Unit, Upjohn Co., Kalamazoo, Mich.). *Recent Prog Neurol Surg, Proc Symp Int Congr Neurol Surg, 5th, Tokyo, Oct 7-13, 1973* (Amsterdam: Excerpta Medica, 1974) pp. 116-122. (32 references)

6065 IMMUNOSUPPRESSIVE CHEMOTHERAPY AND MALIGNANCY. *(E.)* Schwartz, R. S. (N. Engl. Med. Cent. Hosp., Boston, Mass.). *Transplant Proc* 6(4/Suppl. 1):45-48, 1974. (11 references)

6066 CROSS-REACTIVE ANTIGENS, CANCER, AND TRANSPLANTATION. *(E.)* Rapaport, F. T. (New York Univ. Med. Center, N.Y.). *Transplant Proc* 6(4/Suppl. 1):39-44, 1974. (44 references)

6067 AFLATOXIN. *(E.)* Butler, W. H. (Med. Res. Counc. Lab., Carshalton, England). In: *Mycotoxins* edited by I. F. H. Purchase. New York, Elsevier Scientific Publishing Co., 1974, pp. 1-28. (133 references)

6068 OCHRATOXIN A AND RELATED METABOLITES. *(E.)* Harwig, J. (Food Res. Lab., Health Protection Branch, Dep. Natl. Health and Welfare, Tunney's Pasture, Ottawa, Canada). In: *Mycotoxins* edited by I. F. H. Purchase. New York, Elsevier Scientific Publishing Co., 1974, pp. 345-367. (111 references)

6069 LEUKEMIA IN PREGNANCY. *(E.)* McLain, C. R., Jr. (Univ. Cincinnati Coll. Med., Ohio). *Clin Obstet Gynecol* 17(4):185-194, 1974. (24 references)

6070 TUMOURS OF THE GENITALIA. *(E.)* Huffman, J. W. (No affiliation). *Clin Obstet Gynaecol* 1(3):663-682, 1974. (28 references)

6071 GLUCAGONOMA AND A-CELL HYPERPLASIA. *(E.)* Croughs, R. J. M. (No affiliation). *Clin Gastroenterol* 3(3):609-619, 1974. (28 references)

6072 SEXUAL ACTIVITY AND ITS CONSEQUENCES IN THE TEENAGER. *(E.)* Russell, J. K. (No affiliation). *Clin Obstet Gynaecol* 1(3):683-698, 1974. (38 references)

6073 CURRENT CONCEPTS IN LUNG CARCINOGENESIS. *(E.)* Burrascano, J. J. (Sea View Hosp., Staten Island, N.Y.). *NY State J Med* 74(2):350-355, 1974. (64 references)

6074 ENVIRONMENTAL CARCINOGENS: IS THERE A THRESHOLD OF EXPOSURE? *(E.)* Claus, G. (Univ. Vienna, Austria). *Clin Toxicol* 7(5):497-508, 1974. (No references)

6075 CARCINOMA OF THE CERVIX AND HERPES HOMINIS (SIMPLEX) TYPE 2 VIRUS INFECTION. *(E.)* Joncas, J. H. (Inst. Microbiol. Hyg. Montreal, Canada). *Rev Can Biol* 33(3):223-228, 1974. (30 references)

6076 SOME POSSIBILITIES THAT BIOLOGICAL SIGNALS MAY REGULATE CELL PROLIFERATION. *(Ger.)* Popp, F. A. (Radiol. Cent., Univ. Marburg, W. Germany). *Arch Geschwulstforsch* 44(4):295-306, 1974. (31 references)

6077 THE MUTAGENIC EFFECT OF X-RAYS. I. THE OCCURRENCE OF MUTATIONS AND THEIR EFFECTS. *(Ger.)* Stephan, G. (Dep. Radiat. Hyg., Fed. Health Service, Berlin, W. Germany). *Roentgenblaetter* 27(2):75-81, 1974. (10 references)

6078 ENDOGENOUS SYNTHESIS OF CARCINOGENIC AZIRIDINES (ETHYLENE IMINES). *(Ger.)* Bicker, U. (Med. Acad., Erfurt, East Germany). *Arch Geschwulstforsch* 44(4):312-318, 1974. (24 references)

6079 HEALTH HAZARDS CAUSED BY ASBESTOS: THE POSSIBILITIES OF GENERAL AND INDUSTRIAL EXPOSURE. *(Ger.)* Sarrazin, W. (Gauting Cent. Hosp., Upper Bavaria, W. Germany). *Prax Pneumol* 28(7):370-388, 1974. (68 references)

6080 IMMUNE SURVEILLANCE IN CANCER. *(Ger.)* Schneeweiss, U. (Cent. Inst. Cancer Res., Robert Rossle Clin., Berlin, E. Germany). *Z Exp Chir* 7(4):204-215, 1974. (57 references)

6081 NOTES ON THE ANALYSIS OF SPECIFIC ANTIGENICITY OF TUMOR CELLS BY USING PROTECTINS AND LECTINS. *(Ger.)* Prokop, O. (Inst. Forensic Med., Humboldt Univ., Berlin, E. Germany) A. Graffi, P. Luther, C. Gauwerky and G. Uhlenbruck. *Dtsch Gesundheitswes* 29(49):2328-2333, 1974. (38 references)

6082 IS A PREDISPOSITION TO LUNG CANCER A RESULT OF GENETIC VARIABILITY? *(Ger.)* Schmidt, R. (German Cancer Res. Cent., Heidelberg, West Germany). *Dtsch Med Wochenschr* 99(38):1887-1888, 1974. (16 references)

6083 CHRONIC ARSENIC POISONING AND ITS INDEMNIFICATION: DELAYED DEVELOPMENT OF CUTANEOUS ARSENISM AND ARSENIC-INDUCED CANCERS. *(Fr.)* Texier, L. (St. Andre Hosp., Bordeaux, France), P. L'Epee, H.-J. Lazarini, G. Ducombs, J. Doignon, S. Larcebau and M.-J. Miegeville. *Bordeaux Med* 7(10):1539-1544, 1974. (No references)

84 THE CHEMICAL DYNAMICS OF MUTAGENESIS AND CARCINOGENESIS: I. A CHEMICAL-KINETIC NETIC MODEL OF DOSE-RESPONSE RELATIONSHIPS. *(E.)* andler, J. LR. (Dep. Biochem., Oklahoma State Univ., illwater). *Mutat Res* 25(2):169-178, 1974.

descriptive model is presented which allows, in incipile, a direct approach to the problem of chemi- l influences on mutation rates. The model is based the physical-chemical principles which relate the tes and equilibria of changes associated with chem- al processes to energy changes. This approach of- rs the advantage of relating directly to measurable antities associated with the chemical mutagenesis. ree points can be deduced regarding the initial emical process involved in a typical microbial mu- tion experiment: at time zero, the concentration the drug inside the cell is zero, and as an ex- nential function of time, a relationship between e concentration inside and outside the cell is es- blished; each DNA base occupies a unique chemical vironment which differs from that of any other base the polymer; and the different molecular environ- nts in which the DNA is found within the organism st affect its chemical reactivity. This simple del describing the relationship between the DNA kylation products and the number of chemical muta- ions suggests that the number of induced chemical tations must increase in a linear manner with the mber of molecules of the mutagen exterior to the ganism. This model forms the basis for sets of inear and nonlinear differential rate equations ich quantitatively describe the dose-response re- ationship as a function of the exposure time and posure concentration. A classification of chemical tagens is proposed which appears useful for quan- itative comparisons; according to this system, the mpounds are classified as intrinsic alkylating gents, chemically activated mutagens, enzymatically ctivated alkylating agents, inorganic and miscella- eous covalent reactants, and miscellaneous reagents. ne model described is applicable to chemical carcin- genesis in so far as mutation induction is rate- imiting in cancer induction.

)85 INTRANUCLEAR DISTRIBUTION OF DNA REPAIR SYNTHESIS INDUCED BY CHEMICAL CARCINOGENS R ULTRAVIOLET LIGHT IN HUMAN DIPLOID FIBROBLASTS. *E.)* Harris, C. C. (Natl. Cancer Inst., Bethesda, d.), R. J. Connor, F. E. Jackson and M. W. Lieber- an. *Cancer Res* 34(12):3461-3468, 1974.

he intranuclear distribution of DNA repair synthesis as measured by thymidine-^{3}H incorporation) was stud- ed in human fibroblasts following exposure to a iverse group of direct-acting chemical carcinogens nd UV radiation. Confluent, non-S-phase human di- loid fibroblasts (WI-38) showed autoradiographic vidence of DNA repair synthesis following exposure o *N*-methyl-*N*-nitrosourea, 7-bromomethylbenz(a)an- hracene, *N*-acetoxy-*N*-acetylaminofluorene, or UV ight (254 nm) and subsequent labeling with thymi- ine-^{3}H. The intranuclear distribution of DNA re- air synthesis in individual cells was nonrandom, .g., it did not fit a random model (Poisson distri- ution). The autoradiographic grain density near the nuclear envelope ("outer shell") was compared with the density in the central nuclear area ("inner core"). When the grain densities were corrected for the greater packing of DNA in the outer shell, there was approximately a 2-fold increase in the grain densities of the inner core compared with the outer shell. By electron microscopy, the DNA in the inner core was primarily euchromatic, while that in the outer shell was primarily heterochromatic. No alterations in either the ultrastructure of the heterochromatin or its peripheral distribution were observed in cells undergoing DNA repair synthesis. The major ultrastructural change was segregation of the nucleolar granules and fibrils in the cells exposed to N-acetoxy-2-acetylaminofluorene, UV light, or 7-bromomethylbenz(a)anthracene. The results indicate that carcinogen-induced chromatin rearrangement is probably not the cause of the observed nonrandom distribution of repair synthesis.

6086 THE *IN VIVO* AND *IN VITRO* EFFECTS OF AFLATOXIN B_1 ON RAT LIVER AND SPLEEN ACID DEOXYRIBONUCLEASE. *(E.)* Pitout, M. J. (South African Med. Res. Counc., Pretoria), P. G. Kemff and J. C. Schabort. *Int J Biochem* 5(3):262-270, 1974.

The *in vivo* effect of the hepatocarcinogen, aflatoxin B_1, on the activity of rat liver acid DNase was studied along with its *in vitro* effect on rat spleen acid DNase. In the *in vivo* experiments, aflatoxin B_1 (1.5 mg/kg day) was administered intragastrically for 1-7 days to 15 male Wistar-derived rats. In the *in vitro* experiments, acid DNase was isolated from the spleens of male and female Wistar-derived rats, purified, and incubated with aflatoxin B_1. The acid DNase in both experiments was analyzed using polyacrylamide gel electrophoresis, electrofocusing, and Sephadex gel-filtration. In the *in vivo* experiments, aflatoxin B_1 dramatically increased the specific activity of the rat liver acid DNase, while in the *in vitro* experiment, it had no direct activating effect on the splenic enzyme extract. Polyacrylamide gel electrophoresis and electrofocusing revealed that the acid DNase preparations derived from the rat spleens were pure and homogeneous, with a molecular weight of 36,000 and an isoelectric point of 10. With regard to the relationship between aflatoxin B_1 and the observed increase in activity of the liver acid DNase, it is suggested that the toxin itself or a metabolite might interact in some way with the activity of the enzyme, possibly *via* a natural protein inhibitor of the enzyme. It is also possible that the toxin or a metabolic derivative might have a deleterious effect on the lysosomal membranes, thereby resulting in a leakage of acid DNase.

6087 METABOLIC CHANGES CAUSED BY AFLATOXIN B_1 AND EFFECTS ON NUCLEIC ACID AND PROTEIN SYNTHESIS. *(Fr.)* Moule, Y. (Inst. Sci. Res. Cancer, Villejuif, France). *Ann Nutr Aliment* 28(4): 375-384, 1974.

Deviations, triggered by a single dose of aflatoxin B_1 (1 mg/kg), in RNA, DNA and protein synthesis of rat liver were studied to demonstrate how a specific fundamental biochemical procedure can uncover toxicity of a substance and elucidate its mechanism of action. The inhibition of RNA synthesis seen upon administration of C^{14}-orotic acid, and the fact that RNA polymerase of isolated rat liver nuclei is strongly inhibited when treated *in vivo* with aflatoxin demonstrate that the transcription systems, located in the nucleolus and in the nucleoplasm, have been affected by the toxin. Aflatoxin also inhibits synthesis of liver proteins. A biphasic effect and intense early inhibition are observed. The inhibition of protein synthesis is due to the alteration of RNA synthesis by the mycotoxin. The early effect of aflatoxin is interpreted as a manifestation of toxicity and led to the hypothesis that aflatoxin acts directly on the polysomes. Inhibition of DNA synthesis was proven by inhibition of replication.

6088 THE EFFECT OF ALFATOXIN B_1 ON THE MATURATION OF RIBOSOMAL AND TRANSFER RNA IN MONKEY KIDNEY (CV-1) CELLS IN CULTURE. *(E.)* Garvican, L. (Univ. Coll. Hosp. Med. Sch., London, England) and K. R. Rees. *Chem Biol Interact* 9(6):429-434, 1974.

The effects of aflatoxin B_1 on the maturation of ribosomal and transfer RNA in monkey kidney (CV-1) cells were studied *in vitro*. Monolayer cell cultures were labeled with (5-^{3}H)uridine and (2-^{14}C)uridine and incubated with 40 μg/ml of aflatoxin B_1. The nucleolar and cytoplasmic RNA were extracted after various chase periods and analyzed by polyacrylamide gel electrophoresis. Aflatoxin B_1 inhibited the maturation of rRNA by causing a build-up of 32S RNA in the nucleolus which was associated with a reduced output of 28S rRNA into the cytoplasm. Processing of the 18S rRNA appeared normal. Aflatoxin also delayed the maturation process of tRNA, although the data gave no indication of the point in this process at which the toxin was interfering. The toxin may have interrupted the enzymatic cleavage involved in the maturation process by interaction with the particular RNA involved or by interaction with the enzymes involved in the cleavages. The results also suggest that aflatoxin B_1 would be likely to disrupt the maturation process of mRNA.

6089 SIMILITUDES AND DIFFERENCES IN THE REACTIVITY OF THE LIVER CELL CHROMATIN TO THIOACETAMIDE AND HYDROCORTISONE. ELECTRON MICROSCOPIC AND CYTOCHEMICAL QUANTITATIVE DATA CONCERNING THE CHROMATIN CONDENSATION-DISPERSION CYCLE. *(E.)* Moraru, I. (Victor Babes Inst. Pathol. Med. Geneti, Bucharest, Romania), C. T. Dragomir, E. Tomescu, C. Cotutiu and D. Ungureanu. *Pathol Eur* 9(4):317-323, 1974.

The effects of the *in vivo* administration of thioacetamide (TAA) and/or hydrocortisone on the ultrastructure of the liver chromatin and levels of nuclear RNA were studied using electron microscopy and cytophotometrical techniques. TAA (150 mg/kg) and/or hydrocortisone hemisuccinate (100 mg/kg) were injected into Wistar rats and the livers were excised after intervals of 1-6 hr. TAA and hydrocortisone, both alone and in combination, produced increases in the mean nuclear volume, the greatest increases being produced by hydrocortisone alone. TAA administration was also accompanied by an increase in the chromatin RNA concentration and a maximum 3-fold increase over basal levels was noted after 3 hr, after which the concentration diminished. Under the influence of hydrocortisone, the chromatin RNA concentration increased more slowly over a 4-hr interval. After the administration of both drugs, a maximum 4-fold increase over basal levels was seen after 3 hr. The RNA increase began 1 hr after drug treatment, during the period when the chromatin alterations were particularly intense. At 1-2 hr after TAA treatment, dense chromatin was no longer observed; this was followed by marked heterochromatinization, especially of the envelope chromatin and nucleolus-associated chromatin. Hydrocortisone produced a total dispersion of the dense chromatin within 1 hr, which was not followed by condensation within the interval studied. After combined drug administration, the chromatin aspect was that dictated by hydrocortisone. The results suggest that carcinogens, at least TAA, favor certain DNA and chromatin lesions by chromatin decondensation phenomena and rapid chromatin recondensation might locally block the repair system permitting an accumulation of errors in the cellular genome.

6090 INCREASED HALF-LIFE OF RAT LIVER RNA FOLLOWING DIETARY ADMINISTRATION OF 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE AND/OR CHLORAMPHENICOL. *(E.)* Crowther, C. E. (Dep. Pathol., Univ. Melbourne, Parkville, Australia) and J. M. Blunck. *Res Commun Chem Pathol Pharmacol* 9(1):97-106, 1974.

The half-life of rat liver RNA was measured in young male Sprague-Dawley rats pair-fed a control diet and diets containing 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB) and/or 2% chloramphenicol (CAP). The half-life of the liver RNA was determined by estimating the rate of loss of radioactivity from RNA labeled *in vivo* by orotic acid-6-^{14}C. The half-life value in the control rats was 4.262 days, that in the CAP-fed animals was 4.734 days, that in the 3'MeDAB-fed animals was 4.732 days and that in the 3'MeDAB plus CAP-fed animals was 4.931 days. The differences between the values in the control and carcinogen-fed rats were significant. There was a progressive loss of liver RNA in the rats maintained on the control diet for 4, 11, and 18 days. A similar loss was noted in the 3'MeDAB-fed rats, but the loss was less extensive when both 3'MeDAB and CAP were given. When CAP alone was administered, a small increase in total liver RNA was noted during the first ten days, followed by a decrease at 18 days. The results suggest that the observed decreases in total liver RNA were due primarily to decreased rates of RNA synthesis and that the rate of synthesis was more important than the rate of degradation in maintaining the amount of liver RNA. The data also suggest that azo dye feeding increases the half-life of RNA by a reduction in the activity of liver RNase.

6091 MALIGNANT TRANSFORMATION OF RAT LIVER PARENCHYMAL CELLS BY CHEMICAL CARCINOGENS IN TISSUE CULTURE. *(E.)* Katsuta, H. (Inst. Med. Sci., Univ. Tokyo, Japan) and T. Takaoka. *Biochem Dis* 4(Part B):473-481, 1974.

Investigations of the *in vitro* malignant transformation of rat hepatocytes by dimethylaminoazobenzene (DAB) and 4-nitroquinoline-1-oxide (4NQO) are described. Several strains of liver parenchymal cells derived from normal rat tissue have been transformed in tissue culture by DAB (up to 20 γ/ml) or 4NQO (3.3×10^{-6} M one to several times). The transformed lines produce tumors upon back-transplantation into rats and proliferate rapidly when regrown in culture. 4NQO-treated tumor cells have a decreased modal chromosome number (43) and do not agglutinate with concanavalin A unlike control hepatocyte cultures which have undergone spontaneous transformation. Survival times of rats inoculated with successive passages of 4NQO-treated cells progressively shorten whereas those of rats injected with spontaneously transformed cells do not. Neuraminidase-treated, 4NQO transformed hepatoma cells differ from spontaneously transformed cells in electrophoretic mobility, a technique which has proven to be a much quicker means of detecting malignant transformation than waiting for the results of back transformation.

6092 ASPECTS OF HEPATOCARCINOGENESIS. PART V. ULTRASTRUCTURAL MORPHOLOGY OF EARLY HEPATOCELLULAR CARCINOMAS. *(E.)* Timme, A. H. (Dep. Pathol., Univ. Cape Town, South Africa). *S Afr Med J* 48(31): 1335-1337, 1974.

Six hepatocellular nodules which arose in the cirrhotic livers of rats fed p-dimethylaminoazobenzene (p-DAB) were studied by light and electron microscopy. The animals were killed 14 to 20 weeks after commencement of administration of the carcinogen. Serial sections, examined under the light microscope, showed well-differentiated liver cells in four of the nodules which were non-invasive. The remaining two nodules were similar but showed limited signs of infiltration into adjacent parenchyma. With the electron microscope, the cells of the nodules appeared well differentiated with normal microbodies. The cells contained little glycogen except for a few "membrane glycogen arrays." Since all six nodules were indistinguishable from each other under the electron microscope even though two showed signs of infiltration of adjacent tissue, it was concluded that all six nodules were neoplastic. Conclusive proof of the neoplastic nature of the non-invasive nodules would require transplantation to other animals.

6093 CHOLANGIOFIBROSIS INDUCED BY SHORT-TERM FEEDING OF 3'-METHYL-4-(DIMETHYLAMINO)AZOBENZENE: AN ELECTRON MICROSCOPE OBSERVATION. *(E.)* Terao, K. (Res. Inst. Chemobiodynamics, Narashino, Japan) and M. Nakano. *Gann* 65(3): 249-260, 1974.

The ultrastructure and histogenesis of cholangiofibrosis induced by 3'-methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) was studied in 130 male Wistar rats. Feeding with a diet containing 3'-Me-DAB (0.06%) for 2-4 weeks followed by a stock diet for 2 to 82 weeks induced cholangiofibrosis in virtually every animal. Rats fed the treated diet for 12 weeks had cirrhosis, nodular hyperplasia, atypical proliferation of hepatocytes, adenoma, and cholangiofibrosis; and those on the diet for 16 weeks had hepatomas also. Rats treated for one week showed an increase in the agranular endoplasmic reticulum and segregation in the nucleoli of hepatocytes. Typical cholangiofibrosis appeared occasionally after only two weeks on the treated diet. Biliary duct cells, fibroblasts and macrophages and the lining epithelium of the cholangiofibrosis were studied by electron microscopy. After cessation of the feeding of the carcinogen, lesions of cholangiofibrosis continued to grow for up to 25 weeks. Hyalinization of the lesion was seen from eight weeks after the treated diet was stopped, but the newly proliferated lining epithelial cells at the peripheral zone of the hyalinized lesions were always seen. Cholangiofibrosis areas showed a typical intestinal cell metaplasia including goblet cells, enterochromaffin cells, and Paneth cells. It is suggested that cholangiofibrosis may be an autonomous response and that the epithelio-mesenchymal interactions may play an important role in the histiogenesis of these metaplasia.

6094 EFFECTS OF CYCLOPHOSPHAMIDE ON SOMATIC CELL CHROMOSOMES IN RATS. *(E.)* Payne, H. G. (Dep. Pathol. Toxicol., Mead Johnson Res. Cent., Evansville, Ind.), L. W. Nelson and J. H. Weikel, Jr. *Toxicol Appl Pharmacol* 30(3):360-368, 1974.

The acute, subacute, and chronic effects of cyclophosphamide on the somatic cell chromosomes of rats were studied. In the acute and subacute studies, male Sprague-Dawley rats were given 1, 3, or 9 mg/kg cyclophosphamide; in the acute study, the chemical was administered orally by intubation, and in the subacute study, it was given p.o. daily for five days. Colcemid (4 mg/kg, i.p.) was given two hr before sacrifice. In the chronic study, McCollum rats were given 3 mg/kg/day of cyclophosphamide for four generations. The mitotic index in the bone marrow cells was severely depressed 24 hr, but not 6 or 48 hr, after a single dose of cyclophosphamide. The number of chromosome breaks was greater in the two higher dosage groups. Similar results were obtained in the subacute study. In the multigeneration study, a dose-related increase in the number of chromosomal aberrations was observed. Two of 237 animals examined had large numbers of dicentric forms, but there were no distinct or consistent differences in the chromosomal aberrations as a function of sex or generation. The results suggest that the chromosomal aberrations may have been an expression of a direct cytotoxic effect rather than a mutagenic effect since there was no increase in the number of cells with aberrations in successive generations.

6095 PROPERTIES OF RIBOSOMES AND RNA SYNTHESIZED BY *ESCHERICHIA COLI* GROWN IN THE PRESENCE OF ETHIONINE. III. METHYLATED PROTEINS IN 50 S RIBOSOMES OF *E. COLI* EA2.

(E.) Alix, J. H. (Lab. Chim. Cell., Inst. Biol. Phys. Chim., Paris, France) and D. Hayes. *J Mol Biol* 86(1):139-159, 1974.

The effects of ethionine on the methylation of the 50 S ribosomal proteins of *Escherichia coli* were studied. *E. coli* EA2 (met^- RC^{rel}) were grown and labeled in complete mineral medium, to which DL-ethionine and (^{14}C)valine were added. Ribosomal subunits from the labeled cells were analyzed by acrylamide gel electrophoresis, and methylation of the 50 S ribosomal proteins was measured *in vitro* and *in vivo*. The 50 S ribosomes prepared from *E. coli* grown in the presence of thionine were functionally abnormal. They contained a full complement of ribosomal proteins (with the possible exception of L6 and L30), but also contained submethylated forms of 23 S ribosomal RNA and of proteins L11, L3, and L5. Following the *in vivo* or *in vitro* methylation of the ethionine ribosomes or the *in vitro* methylation of the free ethionine ribosomal proteins, protein L11 was found to contain three methyl groups in a single $N_\varepsilon,N_\varepsilon,N_\varepsilon$-trimethyllysine residue and an equal number in another unidentified amino acid. Under similar conditions, protein L3 and L5 incorporated approximately 0.6 and 0.4 methyl groups per molecule, respectively. The sites of methylation of these proteins have not been identified. $N_\varepsilon,N_\varepsilon,N_\varepsilon$-trimethyllysine and the unidentified methylated amino acid occurred in proteins extracted from the 50 S ribosomes of *E. coli* EA2 grown under normal conditions. Protein L11 and possibly proteins L3 and L5 of the *E. coli* EA2 50 S ribosomes therefore normally contain these methylated amino acid residues. These results demonstrate the existence of the corresponding ribosomal protein methylases.

6096 METHYL-DEFICIENT MAMMALIAN TRANSFER RNA. II. HOMOLOGOUS METHYLATION *IN VITRO* OF LIVER tRNA FROM NORMAL AND ETHIONINE-FED RATS: ETHIONINE EFFECT ON 5-METHYLCYTIDINE SYNTHESIS *IN VIVO*.

(E.) Jank, P. (Max-Planck-Inst. Biochem., Munich, West Germany) and H. J. Gross. *Nucleic Acids Res* 1(10):1259-1267, 1974.

In order to establish quantitative differences, the homologous methylation of normal and methyl-deficient rat liver tRNA was studied *in vitro*. Rat liver tRNA methylase activity was assayed following hydroxyapatite chromatography with liver tRNA from normal rats and methyl-deficient liver tRNA from ethionine-fed rats. The maximum difference in homologous methylation occurred in the presence of cadaverine, but a lesser difference was observed in magnesium or magnesium plus cadaverine incubants. The methylase endogenous tRNA fractions were used for preparative homologous methylation of added normal and methyl-deficient tRNA in the presence of 30 mM of cadaverine. Two dimensional thin layer chromatography and autoradiography were used to characterize and quantitate the methylated mononucleotides resulting from RNAase T_2 digestion of ^{14}C-methylated tRNA; the major products were n^5C and m^1A. Ethionine feeding caused complete m_2^6A- and partial m^5C-deficiency in liver tRNA, but in the presence of cadaverine, methyl-deficient tRNA accepted twice as many methyl groups as the normal tRNA. The author proposes that this increased efficiency may be a consequence of hydroxyapatite chromatography or of the preferential binding of endogenous methyl-deficient tRNA to tRNA methylase.

6097 MUTAGENESIS IN CULTURED HUMAN DIPLOID CELLS. II. CHEMICAL INDUCTION OF 8-AZAGUANINE-RESISTANT MUTATIONS.

(E.) Kuroda, Y. (Natl. Inst. Genet., Shizuoka, Japan). *Idengaku Zasshi* 49(6):389-398, 1974.

Human diploid cells were treated *in vivo* with ethyl methanesulfonate (EMS) and cultured in selection medium containing 8-azaguanine (8AG), and the effects of the inoculum dosage and the mutation expression time on the frequency of 8AG-resistant mutations were examined. When the cells were treated with 10^{-2} M EMS for 2 hr and selected in medium containing 10 µg/ml of 8AG, the induced mutation frequency was highest following the inoculation of 2.5×10^5 cells; this frequency was not statistically significant. When EMS-treated cells were selected with 30 µg/ml of 8AG, the highest induced mutation frequency was obtained on inoculation of 10^5 cells. The mutation frequency was higher when 10^5 cells treated with EMS were cultured in normal medium for 48 hr (mutation expression time) before selection with 8AG than when longer or shorter mutation expression times were allowed. This mutation expression time of 48 hr corresponded to the time during which about 1/2 of the cell population treated with EMS divided once. Microscopic examination of the cell colonies showed that human diploid cells grown with or without EMS did not all grow exponentially, and that in some cases after each cell division only one daughter cell continued to divide. The results suggest that DNA replication is necessary for the phenotypic expression of gene mutations in single-stranded DNA.

6098 *IN VITRO* STUDIES ON THE EFFECT OF HYDRAZINE ON THE MORPHOGENESIS OF CHICK EMBRYOS AND THE MECHANISM OF ITS ACTION.

(E.) Telang, N. T. (Dep. Zool., Univ. Poona, India) and L. Mulherkar. *Oncology* 30(6):529-541, 1974.

The effect of hydrazine on embryos of White Leghorn chicks cultivated *in vitro* was investigated to determine its mode of action. Hydrazine-treated embryos, when grown in Pannet-Compton's saline for 18-20 hr at 37.5 ± 1 C showed abnormalities which were predominantly restricted to the brain, neural tube, optic vesicles and somites. The differentiation of the heart was also affected, but to a lesser degree. When the embryos were subsequently treated with different amino acids, these showed a certain degree of specificity. Not all the amino acids used were equally effective in bringing about a reversal of the hydrazine-induced malformation. Predominantly affected were the amino acid conversions which depend on transmethylation reactions. The hydrazine-induced abnornalities were effectively reversed by glycine but not by serine and by methionine but not by homocysteine, suggesting that the amino acid conversions which were mediated by folic acid were impaired in the hydrazine-treated embryos. From among the nucleic acid precursors which were used for subsequent treatment, thymidine effectively reversed the inhibitory effects of hydrazine while uridine did not. Since other precursors such as orotic acid and adenylic acid were not effective in bringing about the reversal, the results obtained with thymidine and uridine can be explained on the basis of a block in normal methylation reactions brought about by hydrazine. Folic acid itself, as well as related substances such as p-aminobenzoic acid, choline, vitamin B_{12} and vitamin C were also quite effective in reversing the inhibitory effects of hydrazine. The mode of action of hydrazine was related to the possible inhibition of normal one-carbon and methyl group metabolism by a deficiency of folic acid, induced by hydrazine. The results suggest the existence of a close parallelism between biochemical lesions due to folic acid deficiency noticed in the microbial systems and the chick embryo which has been exposed to hydrazine.

6099 CARCINOGENICITY OF DIFFERENT POLYCYCLIC HYDROCARBONS IN THE RESPIRATORY TRACT OF HAMSTERS. *(E.)* Sellakumar, A. (Eppley Inst. Res. Cancer, Univ. Nebraska, Omaha) and P. Shubik. *J Natl Cancer Inst* 53(6):1713-1719, 1974.

The chemical activities of benz[*a*]anthracene, benzo[*b*]fluoranthene, dibenz[*a*,*h*]anthracene, dibenzo[*a*,*i*]pyrene (DB[*a*,*i*]P) and pyrene were tested in the respiratory tracts of male Syrian golden hamsters. The carcinogens were administered intratracheally with equal amounts of Fe_2O_3 at weekly intervals. Hamsters receiving benz[*a*]anthracene (30 doses of 0.5 mg or 15 doses of 3.0 mg), benzo[*b*]fluoranthene (30 doses of 0.05 mg), dibenz[*a*,*h*]anthracene (24 doses of 0.05 mg), and pyrene (30 doses of 3 mg) did not show any respiratory tract tumors. In a group of 47 hamsters exposed to 30 doses of 0.5 mg benzo[*b*]fluoranthene one had a papilloma of the trachea. In a group of 46 animals receiving 30 doses of dibenz[*a*,*h*]anthracene (0.25 mg) two had adenocarcinomas. Of the hamsters receiving 24 doses of DB[*a*,*i*]P (0.5 mg), 39 of 44 (89%) developed respiratory tract tumors while of those receiving four doses of 2.0 mg, 16 of 34 (47%) developed tumors. The majority of these tumors were squamous cell carcinomas. In the higher dose group 30 of 37 developed squamous carcinomas in the bronchus. Respiratory tract carcinogenicity of DB[*a*,*i*]P is greater than that of benzo[*a*]pyrene, a well established carcinogen.

6100 THE METABOLISM OF POLYCYCLIC HYDROCARBONS BY CULTURED HUMAN LYMPHOCYTES. *(E.)* Booth, J. (R. Cancer Hosp., London, England), G. R. Keysell, K. Pal and P. Sims. *FEBS Lett* 43(3):341-344, 1974.

The metabolism of three polycyclic hydrocarbons (benz(a)anthracene, 7-methylbenz(a)anthracene and benzo(a)pyrene) was studied in cultured human lymphocytes. Chromatographic examination of incubation mixtures containing cultured lymphocytes, NADPH, and [^{3}H]benz(a)anthracene (2 μg) showed two bands of radioactivity that had the chromatographic properties of the 5,6- and 8,9-dihydrodiols of benz(a)anthracene. In similar experiments with 7-methylbenz(a)anthracene, radioactive bands were found coincident with the dihydrodiols of the 7-methylbenz(a)anthracene. The radioactivity profile of thin-layer chromatograms from experiments using benzo(a)pyrene showed one peak with the chromatographic properties of 3-hydroxybenzo(a)pyrene and others with those of the 4,5-, 8,9- and 10,11-dihydrodiols of benzo(a)pyrene. None of the metabolites of any of the hydrocarbons was detected when lymphocytes were omitted from the reaction mixtures, thus showing that the lymphocytes converted the hydrocarbons into dihydrodiols that are also formed by rat liver preparations. The presence of epoxide hydrase was demonstrated when a radioactive product that was chromatographically indistinguishable from trans-5,6-dihydro-5,6-dihydroxybenz(a)anthracene was formed after incubating lymphocytes with [^{3}H]benz(a)anthracene 5,6-oxide. Cultured human lymphocytes also contained glutathione S-epoxide transferase activity because [^{3}H]benz(a)anthracene 5,6-oxide reacted with GSH in the presence of the soluble cell fraction to yield a radioactive product that was coincident on chromatograms with S-(5,6-dihydro-6-hydroxybenz(a)anthracene-5-yl)glutathione.

6101 FLAVONES AND POLYCYCLIC HYDROCARBONS AS MODULATORS OF ARYL HYDROCARBON [BENZO(a) PYRENE] HYDROXYLASE. *(E.)* Wiebel, F. J. (Natl. Cancer Inst., Bethesda, Md.), N. P. Buu-Hoi, M. G. Stout and W. S. Burnham. *Biochem Dis* 4(Part A): 249-270, 1974.

Several flavones and polycyclic hydrocarbons were studied for their *in vitro* modifying effects on microsomal aryl hydrocarbon hydroxylase (AHH) activity from untreated and methylcholanthrene (40 mg/kg)- or phenobarbital (80 mg/kg)-stimulated Sprague Dawley rats. Three major groups of flavone derivatives were distinguished: 1) the 5,6- and 7,8-benzoflavones and their more hydrophobic derivatives which inhibit the stimulation of enzyme activity but which increase or have no effect on activity of the unstimulated constitutive enzyme; 2) flavones modified in the 4'-position (e.g., 4-hydroxylated benzoflavones) which decrease the induced and constitutive enzymes to similar extents; and 3) polyhydroxyflavones which inhibit the constitutive enzyme to a greater extent than the induced enzyme. Two polycyclic hydrocarbons, 9-chloro-7H-dibenzo(a,g)-carbazole and 6-aminochrysene, both potent inhibitors of AHH, affected the constitutive and induced enzyme similarly to the compounds in the first and second groups, resp. Two different forms of rat microsomal AHH were identified from studies on the effect of 7,8-benzoflavone on enzyme activity: 1) Form I, which is stimulated by flavone, is found in the liver of newborn rats, predominates in normal adult rat liver, and is inducible by phenobarbital; 2) Form II, inhibited by 7,8-benzoflavone, comprises most of the AHH activity of nonhepatic tissues and normal female adult rat liver and is inducible by polycyclic hydrocarbons.

6102 THE EFFECT OF BENZO(*A*)PYRENE ON THE BASAL AND ISOPROTERENOL-STIMULATED LEVELS OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE IN MOUSE EPIDERMIS. *(E.)* Verma, A. K. (Sch. Biol. Sci., Flinders Univ. South Australia, Bedford Park) and A. W. Murray. *Cancer Res* 34(12):3408-3413, 1974.

A study was made of the effect of benzo(*a*)pyrene on the accumulation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in response to isoproterenol in the skin of female Swiss albino mice (three months old). Benzo(*a*)pyrene was applied to the shaved back skin of the animals as a 1% solution in acetone; shaved areas of individual mice were treated with two drops of the benzo(*a*)pyrene solution (equivalent to 0.05-0.06 mg of hydrocarbon/mouse). The level of cyclic AMP was measured ten min after i.p. injection of isoproterenol (10 nM/g) or 0.9% NaCl solution. *In vitro*, isoproterenol was added to Bullough's medium containing excised dorsal skin. Three applications of benzo(*a*)pyrene to the dorsal skin of mice over a nine-day period caused a three-fold increase in the basal level of cyclic AMP. However, this application of benzo(*a*)pyrene did not affect the level of cyclic AMP in the ears. A decrease was observed in the *in vivo* response of epidermal cyclic AMP level to isoproterenol. Incubation of untreated mouse skin pieces for five min with 10^{-4} M isoproterenol caused a six-fold increase in the level of cyclic AMP. However, the cyclic AMP levels of skin pieces from mice treated with benzo(*a*)pyrene were not significantly increased by isoproterenol. After application of acetic acid or croton oil to mouse skin, a decrease in the isoproterenol stimulation of cyclic AMP occurred. This indicated that the effect was not carcinogen specific. The decrease in response was not due to an increase in the metabolism of isoproterenol.

6103 MALIGNANT TRANSFORMATION OF HAMSTER EMBRYO CELLS IN TISSUE CULTURE BY 7,10-DIMETHYLBENZO(C)ACRIDINE. *(Fr.)* Markovits, P. (Curie Found., Paris, France), J. Coppey, D. Papadopoulo, A. Mazabraud and M. Hubert-Habart. *Int J Cancer* 14(2):215-225, 1974.

Hamster embryo cells were treated with 7,10-dimethylbenzo(c)acridine in order to determine its *in vitro* transformation capacity. Three and one-half months later, characteristic tridimensional colonies, containing transformed, fusiform, sarcomatoid cells appeared in the fourth subculture. The cells derived from the subcultures of these colonies produced sarcomas in all inoculated new-born hamsters. An inoculum of 1 x 10^4 cells/animal produced visible tumors in all animals within an average time of 67 days. An increase in the size of the inoculum produced a decrease in the average time until tumor appearance. This study demonstrates that hamster embryo cells directly seeded in Falcon plastic flasks can be successfully used in transformation studies.

6104 CHROMOSOMES OF PRIMARY CARCINOMAS INDUCED BY 7,12-DIMETHYLBENZ(A)ANTHRACENE IN THE RAT. *(E.)* Ahlström, U. (Dep. Oral Surg., Univ. Gothenburg, Sweden). *Hereditas* 78(2):235-244, 1974.

A method was developed for obtaining chromosome preparations from keritinizing squamous cell carcinomas and applied to the study of chromosome aberrations in such tumors. Keratinizing squamous cell carcinomas were induced in Sprague-Dawley rats by the local application of 7,12-dimethylbenz(a)anthracene (DMBA) to the inner surfaces of the auricles. Colchicine (10 µg bodyweight) was injected i.p. 4 hr before killing the rats with ether. Minced tumor pieces were suspended in 5 ml 0.5% trypsin and colchicine and then incubated (37 C, 45 min). Cells were then fixed (acetic acid:0.1N HCl:H_2O [6:1:3]) for 20 min. The chromosomes were then studied in direct preparations of ten primary carcinomas resulting from this treatment. The chromosome variation accompanying the malignant growth was nonrandom, involving six individually recognizable chromosome types. Two of these types were involved frequently, viz. A_2 and C_{12}, the former preponderantly with gains and the latter with losses. The B and D groups were also frequently involved, but generally with gains. The 58 cells karyotyped in the ten preparations contained 42 different karyotypes, 25 in the diploid range, four in the triploid, and 13 in the tetraploid region. Only three cells had one marker each. The chromosome pattern seen in these preparations was of the same type as was previously reported for DMBA-induced leukemias and sarcomas in the rat. These results support the conclusion that the inducing factor, not the tissue of origin, determines the karyotypic evolution in the tumor cells.

6105 THE SPECIFICITY OF CHROMOSOME A2 INVOLVEMENT IN DMBA-INDUCED RAT SARCOMAS. *(E.)* Levan, G. (Inst. Genet., Univ. Lund, Sweden), U. Ahlstrom and F. Mitelman. *Hereditas* 77(2):263-280, 1974.

The chromosomes of 13 fibrosarcomas induced in Wistar/Furth rats by 7,12-dimethylbenz(α)anthracene (DMBA) were studied in direct preparations and in early cultures using conventional and Giemsa banding techniques. Young adult rats were given s.c. 1 mg DMBA dissolved in 0.1 ml tricaprylin. It was found in 12 or the 13 tumors, that added material from chromosome A2 was involved in the stemline karyotypes, either as extra complete A2 or as marker chromosomes containing segments of A2. Through structural analysis of these A2-containing marker chromosomes the significant segment was located on bands in the middle region of A2. The chromosomal analysis of these sarcomas showed that there was a specific involvement of chromosome A2 in the development of DMBA tumors in the rat.

6106 INHIBITION BY CYSTEAMINE-HCL OF ONCOGENESIS INDUCED BY 7,12-DIMETHYLBENZ(A)ANTHRACENE WITHOUT AFFECTING TOXICITY. *(E.)* Marquardt, H. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.), M. D. Sapozink and M. S. Zedeck. *Cancer Res* 34(12):3387-3390, 1974.

The effects of cysteamine-HCl *in vitro* and *in vivo* on 7,12-dimethylbenz(a)anthracene (DMBA)-induced oncogenesis and toxicity were investigated. Twenty-four hr after mouse fibroblasts (M2 clone) were plated, the cells were treated with DMBA (10 μg/ml). Cysteamine-HCl (1 μg/ml) was added to the culture at 20 min before, at the time of, and at five and 24 hr after DMBA addition. Mammary tumors were induced by giving female Sprague-Dawley rats (CD line) i.v. injections of DMBA (15 mg/kg). To induce adrenal necrosis, rats were given DMBA (25 mg/kg) i.v. Lesions of the small intestinal epithelium were also produced. Cysteamine-HCl was given i.p. at a dose of 150 mg/kg at 20 min prior to and five and 24 hr after the administration of DMBA. The results show that cysteamine-HCl, while not affecting DMBA-induced lethality (plating efficiency), markedly reduced the number of DMBA-induced transformed foci. *In vivo*, at four months after treatment with DMBA, 12/18 rats developed a total of 17 mammary tumors, whereas only 4/17 rats receiving DMBA and cysteamine-HCl developed tumors (seven). From 4-11 months, an increase in tumor incidence was observed in both groups of animals. At 11 months, the number of rats with tumors and the total number of tumors in rats treated with cysteamine-HCl and DMBA was equivalent to the tumor incidence observed at four months in those rats given only DMBA. The data showed that cysteamine-HCl prolonged the latent period for tumor development. However, cysteamine-HCl did not influence the DMBA-induced adrenal necrosis and lesions of the small intestinal epithelium. The oncogenic and toxic changes induced by DMBA may be due to two different metabolites; the oncogenic changes may be mediated by a radical.

6107 LINK BETWEEN CARCINOGENICITY AND HEPATIC METABOLISM OF 7,12-DIMETHYLBENZ(α)ANTRACENE.

(E.) Feuer, G. (Dep. Clin. Biochem., Univ. Toronto, Canada) and J. A. Kellen. *Oncology* 30(6): 499-508, 1974.

The relationship between the hepatic metabolism of 7,12-dimethylbenz(α)anthracene (DMBA) and the incidence of mammary tumors was investigated in Sprague-Dawley and Wistar rats. Multiple mammary tumors of different sizes were found in animals treated with 2 mg of DMBA on days 50, 53 and 56 of life. At 57 days, sections from livers of rats treated with DMBA or lipid emulsion showed no apparent histological changes. Rats given 1 mM/g of Coumarin as well as group receiving high levels of Coumarin and DMBA simultaneously, showed discreet signs of focal necrosis and glycogen depletion. The liver damage was transient and reversed to normal 7-14 days after the Coumarin treatment was discontinued. No necrosis was seen in the livers of rats given 0.15-0.4 mM/kg of Coumarin. The administration of DMBA or Coumarin alone and Coumarin and DMBA in combination brought about a significantly reduced Coumarin 3-hydroxylase and glucose 6-phosphate activity at day 57. In the DMBA-treated group, the first tumor appeared at seven weeks; at 100 days 7/9 rats had tumors with an average of two nodules/animal. The exposure to Coumarin before DMBA injections entirely blocked the induction of mammary tumors, so that no palpable nodule was observed. When Coumarin was given after DMBA treatment, no protective action was seen and mammary tumors appeared as in the DMBA-treated group. At 150 days all DMBA-treated rats had developed tumors with an average 2.3 nodules/animal. In the Coumarin and DMBA group only one rat showed one nodule which became apparent at the 20th week. There was no tumor in the Coumarin-treated and control groups. Pregnancy had no effect on tumor formation. The results show that the induction of mammary tumors by DMBA is apparently related to its metabolism in the liver, which can be blocked by Coumarin or reduced in pregnancy.

6108 CHANGE IN THE CONCENTRATION OF FREE RADICALS IN THE TISSUES OF THE INTERNAL ORGANS OF RATS AFTER ADMINISTRATION OF DMBA AND ITS NON-CARCINOGENIC ANALOGUE.

(E.) Petyayev, M. M. (Rostov Oncol. Res. Inst., USSR), V. S. Skotnikov and L. V. Taradin. *Biofizika* 19(1):197-199, 1974.

The electrophrenic respiration (EPR) method was used to study the temporal changes in concentration of free radicals in the tissues of rats treated with 7,12-dimethylbenzanthracene (DMBA). Random bred rats were injected s.c. with 2 mg DMBA or anthracene, a noncarcinogenic analogue of DMBA. The EPR spectra of the tissues were recorded at room temperature with an EPR-2-radiospectrometer. Wave-like fluctuations in the EPR spectra of the liver, lungs, brain, spleen, suprarenal, and thymus tissues were seen after DMBA administration. The curves for the splenic and lung tissue were almost identical after DMBA and anthracene administration, whereas distinct and significant differences between the DMBA- and anthracene-induced curves were seen in the suprarenal, thymus, liver, and brain tissues. The observed differences in the liver tissue may indicate the distinctive bio-antioxidative function of this organ, which responds to the action of the carcinogen more intensely than to that of the noncarcinogenic analogue. Similarly, the observed differences in the brain tissue may be connected with the development of a special adaptive reaction with a high level of bio-antioxidative protection of the nerve tissue.

6109 REACTIONS OF POLYCYCLIC HYDROCARBON EPOXIDES WITH RNA AND POLYRIBONUCLEOTIDES.

(E.) Swaisland, A. J. (R. Cancer Hosp., London, England), P. L. Grover and P. Sims. *Chem Biol Interact* 9(5):317-326, 1974.

RNA, poly(G) and poly (A) were reacted with benz(a)anthracene 5,6-oxide or with 7,12-dimethylbenz(a)anthracene 5,6-oxide, and hydrolysates of the alkylated polymers were examined by a combination of Sephadex LH20 column chromatography and thin-layer chromatography on silica gel. The results show that two RNA products are formed in reactions with benz(a)anthracene, one resulting from reaction with guanine and the other from reaction with adenine. With 7,12-dimethylbenz(a)anthracene 5,6-oxide, six RNA products appeared to be formed, two resulting from reactions with guanine and three from alkylation of adenine; the other product had not been identified. This study supports the view that the purine moieties in nucleic acids may be the principal sites of reaction of polycyclic hydrocarbon epoxides.

6110 EARLY DIFFERENTIAL EFFECTS OF 3-METHYLCHOLANTHRENE AND ITS "K-REGION" EPOXIDE ON MOUSE SKIN. POSSIBLE IMPLICATION IN THE TWO-STAGE MECHANISM OF TUMORIGENESIS. *(E.)* Burki, K. (Med. Coll. Georgia, Augusta), J. E. Wheeler, Y. Akamatsu, J. E. Scribner, G. Candelas and E. Bresnick. *J Natl Cancer Inst* 53(4):967-976, 1974.

The carcinogenic potential of 3-methylcholanthrene (MCA) and MCA-11,12-oxide, administered with or without 1,1,1-trichloro-2-propene oxide (TCPO), in 2-stage skin carcinogenesis system was studied in ten-week-old male BALB/c mice. Repeated percutaneous coadministration of 1.5 μM TCPO with 1.5-3.0 μM MCA increased the carcinogenic effect of the polycyclic hydrocarbon, presumably by inhibition of the hydration of MCA-11,12-oxide, which would increase the effective intracellular concentration of the carcinogeneic epoxide. A single dose of TCPO increased the tumor incidence and shortened the latency period in experiments where MCA was the initiator and phorbol ester was the promoter. MCA-11,12-oxide was only weakly tumorigeneic after either single or repeated topical administration with or without TCPO. Measured morphometrically and cytokinetically, using ^{3}H-thymidine, MCA treatment caused epidermal hypertrophy and hyperplasia, which was absent after treatment with MCA-11,12-oxide. However, the oxide caused a thinning of intrafollicular epidermis due to a decrease in total number of epithelial cells.

6111 EFFECT OF 3-METHYLCHOLANTHRENE ON IMMUNE RESPONSE AND THYMUS WEIGHT OF BUFFALO STRAIN RATS. *(E.)* Silverman, D. A. (Cent. Immunol. Dep. Microbiol., State Univ. New York, Buffalo) and N. R. Rose. *J Natl Cancer Inst* 53(6):1721-1724, 1974.

The 3-methylcholanthrene (MCA) induced increase in autoimmune thyroiditis in Buffalo strain (BUF) rats was studied to determine its effect on immune response. BUF and Lewis strain (LEW) rats were given MCA either orally (0.033 g/100 g chow) starting at four weeks, or i.p. (8 mg MCA/ml in 0.5 ml corn oil) for six days prior to sheep red blood cell (SRBC) immunization. SRBC (1 ml, 20% washed cells) was given i.p. at nine weeks. Response to SRBC was measured using humoral antibody production by a microtiter hemagglutination test; and the formation of plaque-forming cells (PFC), by a modification of the agar plate technique. Complete Freund adjuvant (CFA), was used to sensitize one group of BUF rats (0.2 ml between hind foot pads) at four weeks and twice more during the next six weeks. Another group was sensitized at four weeks with Philip's strain BCG (0.95 ml) suspended in sterile phosphate buffered saline at 1 mg/ml and given s.c. at ten to twenty sites. Skin tests were performed using 0.1 ml purified tuberculin (PPD). At four days after SRBC immunization, humoral antibody response showed higher titers in MCA-treated BUF rats than in untreated BUF rats, and higher titers in all BUF rats than in LEW rats. MCA had no effect on the formation of PFC following SRBC immunization. Reaction to PPD also indicated a failure of MCA to suppress CFA induced delayed hypersensitivity. Skin tests showed MCA to suppress delayed hypersensitivity with respect to induration in the BCG immunized group. The MCA associated loss of thymus and body weight was lessened with BCG immunization. BCG had no effect alone. In BUF rats MCA has a selective effect on some actions attributed to the thymus but does not impede induction of humoral antibodies to a foreign thymus-dependent antigen.

6112 LACTOSE SYNTHETASE INDUCTION BY HORMONES IN NORMAL AND TUMOROUS GR MOUSE MAMMARY TISSUE. *(E.)* Schulein, M. (Fibiger Lab., Copenhagen, Denmark), J. L. Daehnfeldt and P. Briand. *Int J Cancer* 14(3):372-378, 1974.

To analyze the effect of hormones on protein synthesis in hormone-induced GR mouse mammary tumors, a comparative study was made of the A and B protein contents of the hormone-inducible enyzme lactose synthetase in animals of the inbred GR/A strain. The A protein could be induced by estrone (orally administered) and progesterone (s.c.) separately and in combination in the mammary glands of ovariectomized mice. Increased amounts of this protein were observed during pregnancy, when peak levels were recorded in the lactating gland. A 3-fold increase was found in primary tumors as well as in transplanted hormone-dependent and independent tumors, as compared with the level in normal mammary tissue. No significant difference could be demonstrated between A protein activity in the two types of tumors and no further increase was observed in animals with independent tumors following hormone treatment. The B protein could be demonstrated in the normal mammary tissue of intact as well as ovariectomized mice. There was a decrease in the levels of this protein in pregnant animals, while peak values were recorded in lactating animals. A rise in B protein could be induced by estrone in normal mammary tissue, while progesterone blocked its induction. No significant amount of B protein was found in primary tumors or in transplanted hormone-dependent and -independent tumors nor was any increase noted in mice with independent tumors following hormone treatment. It is concluded that the hormonal induction of B protein is repressed in GR mammary tumors. The A protein also is no longer hormone-inducible in these tumors, but its level is higher than that in normal unstimulated mammary tissue.

6113 TRANSFORMATION OF TESTOSTERONE INTO ESTROGENS BY INCUBATED HUMAN BREAST SLICES. *(Fr.)* de Thibault de Boesinghe, L. (Radiother. Clin., Univ. Gand, Belgium), E. Lacroix, W. Eechaute and I. Leusen. *Ann Endocrinol (Paris)* 35(6):691-693, 1974.

In order to determine conversion or lack of conversion of testosterone into estrogens, breast tumor tissue slices obtained from 24 patients were incubated in the presence of testosterone-^{3}H for 2 hr at 37 C. The results showed that all tumors converted testosterone; the degree of conversion varied widely depending on the tumor, but occurred regardless of the quantity of incubated tissue or tumor type (intracanalicular epithelioma, infiltrating epithelioma, carcinoma, breast dysplasia). From

32.5-94.9% of the testosterone was metabolized. The metabolites which were extracted with dichloromethane included: "unidentified metabolites," androstenedione, estrone and estradiol. The formation of androstenedione (1.0-33.8%) and of unidentified metabolites (5.4-34.4%) varied greatly. Furthermore, in 10/24 tumors, testosterone was converted into estrone (0.2-3.9%) and estradiol (0.2-4.0%). In addition, one tumor converted testosterone into estradiol (0.2%), without detectable formation of estrone. The above observations point to the existence of two types of human breast tumors, according to whether they contain or lack the aromatization enzyme capable of converting androgens into estrogens.

6114 ANDROGENS IN BREAST CANCER. II. ENDOMETRIAL ADENOCARCINOMA AND BREAST CANCER IN MARRIED POSTMENOPAUSAL WOMEN.

(E.) Grattarola, R. (Natl. Inst. Study Cure Tumors, Milan, Italy), G. Secreto, C. Recchione and W. Castellini. *Am J Obstet Gynecol* 118(2):173-178, 1974.

The urinary excretion levels of luteinizing hormone (LH or ICSH), testosterone and estrogens (estrone and estradiol-17β) were determined in eight patients with endometrial carcinoma and 17 patients with breast carcinoma and were compared with the levels of normal controls. All patients were married and postmenopausal. In the endometrial cancer patients the range of testosterone excretion level was 10.5 to 31.0 μg/24 hr, which was significantly increased over the control value. The estrogens were not significantly increased; the urinary ICSH concentration varied between 11 and 800 IU. Histological examination of the ovaries revealed the presence in the stroma of large polyhedral or rounded cells that resembled luteinized ovarian theca cells. In eight breast cancer patients, testosterone and estrone estrogen levels were not significantly different from those of the controls, while the urinary ICSH concentrations tended to be lower. In the other breast cancer patients, endometrial histologic examination disclosed glandular hyperplasia. Testosterone excretion levels were markedly increased (20-40 μg/24 hr), while the estrogen and urinary ICSH concentrations were not significantly different from controls. It is inferred that there might be a correlation between interstitial cell hyperplasia and increased androgenic activity, which might be a factor in the endometrial malignancy. The data support the hypothesis that androgens play an important role in the development of endometrial hyperplasia and of endometrial and breast carcinoma.

6115 A HISTOCHEMICAL APPROACH TO THE TOXIC EFFECTS OF LEAD ON THE ACTIVITY OF SOME ENZYMES IN THE KIDNEY OF COMMON GROUND SQUIRREL *FUNAMBULUS PENNANTI.*

(E.) Rana, S. V. S. (Dep. Zool., D. A. V. Coll., Muzaffarnagar, India). *Folia Histochem Cytochem (Krakow)* 12(2):193-196, 1974.

The toxic effects of lead nitrate on the activity of enzymes such as alkaline and acid phosphatases, 5-nucleotidase, lipase, non-specific esterase, and phosphoamidase in the kidney of the common ground squirrel, *Funambulus pennanti* were investigated. A group of 25 squirrels was fed a diet containing 1.0 g of lead nitrate/20 g of feed on alternate days for one month. Microscopic examination of alkaline phosphatase revealed noticeable deviations from the norm in its localization and activity. It was inhibited to a great extent after lead nitrate treatment. There was no activity in the proximal convoluted tubules. The basal epithelial portions demonstrated a positive reaction. The control kidney showed a good activity of the enzyme which appeared in the brush border of the proximal convoluted tubules. No acid phosphatase activity was detected in kidneys of the lead-treated squirrels, whereas in normal animals lysosomes were seen to contain the enzyme. In the experimental animals, 5-nucleotidase was localized in the medullary cells of the kidney; in normal animals this enzyme was in the cortex only. In the experimental animals lipase activity did not occur in the glomeruli as in normal kidneys, but did occur in the convoluted tubules. Nonspecific esterase gave a diffuse reaction product occurring throughout the kidney cortex in lead-treated squirrels. Kidney sections from normal squirrels showed a relatively intense brown staining in the sites of the enzyme activity. The glomeruli were the only esterase-positive site. The cortex of the treated kidneys showed a moderate reaction for this enzyme whereas the medulla was completely negative. The glomeruli were phosphoamidase-positive. In the kidneys of normal squirrels only the glomeruli were enzyme-positive. The results resemble the behavior of these enzymes in the Fanconi syndrome.

6116 DEACYLATION OF CARCINOGENIC 5-NITROFURAN DERIVATIVES BY MAMMALIAN TISSUES.

(E.) Wang, C. Y. (Univ. Wisconsin Med. Sch., Madison) and G. T. Bryan. *Chem Biol Interact* 9(6):423-428, 1974.

The deacylations of N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA), and formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrozide (FNT) by liver, kidney, small intestines, and stomach tissues from female Swiss mice, female Sprague-Dawley rats, male Syrian golden hamsters, and male guinea pigs were studied *in vitro*. The tissues were homogenized and the homogenates incubated with FANFT, NFTA, and FNT. The reaction products were analyzed by thin-layer and gas-liquid chromatography and by mass spectrometry. FANFT was deformylated to 2-amino-4-(5-nitro-2-furyl) thiazole (ANFT), the activity of FANFT formamidase being higher in the liver and small intestines of the mouse, hamster, and guinea pig; and relatively low in the intestines and stomach of the rat. There was no detectable FANFT formamidase activity in the stomach of the mouse or hamster, and neither NFTA nor FNT was deacylated by the rodent tissue homogenates studied. It is suggested that ANFT is a metabolite fo FANFT but not of NFTA; that 2-hydrazino-4-(5-nitro-2-furyl)thiazole may not be a metabolite of FNT; and that the induction of tumors by FANFT, FNTA, and FNT may not be due to a common carcinogenic metabolite, although these chemicals demonstrate similar organ specificities in some of the rodents studied.

6117 REPAIR OF LESIONS INDUCED IN HUMAN LIVER CELL DNA BY N-NITROSO COMPOUNDS AS MEASURED BY THE BROMODEOXYURIDINE PHOTOLYSIS METHOD. *(E.)* Kimble, C. E. (Dep. Nutr. Food Sci., Massachusetts Inst. Technol., Cambridge), P. A. Gorczyca and A. J. Sinskey. *Mutat Res* 24:35-39, 1974.

The DNA repair mechanism in Chang human liver cells previously damaged by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and nitrosomorpholine (NM) was studied. The effect of the folic acid analog, 2-hydroxy-N^{10}-nitrosofolic acid, was also evaluated. Repair of the damage to DNA was estimated by selective photolysis of 5-bromodeoxyuridine (BUdR) containing repaired regions with 313 nm radiation. The liver cells treated with 0.03 μg/ml MNNG and exposed to 313 nm radiation showed eight single-strand breaks (or alkali-labile bonds)/36 x 10^7 daltons. Following incubation for two hr in regular medium the DNA had an M_n of 8 x 10^7 daltons, or four single-strand breaks/36 x 10^7 daltons of starting materials. Thus, during the two hr recovery period the number of observable breaks was reduced by 50%. The folic acid analog caused no significant damage over the controls; it induced three repairable lesions/10^8 daltons at 100 μg/ml. The carcinogen NM caused more damage, producing about 18 breaks/10^8 daltons. Arlacel A (500 μg/ml) inhibited repair synthesis after irradiation. Results indicated significant repair synthesis with MNNG and NM treated cells.

6118 EFFECTS OF INTRAPERITONEAL INJECTIONS OF DIMETHYL- AND DIETHYLNITROSAMINE, ALONE OR SIMULTANEOUSLY ON SWISS MICE. *(E.)* Cardesa, A. (Univ. Nebraska Med. Cent., Omaha), P. Pour, J. Althoff and U. Mohr. *Z Krebsforsch* 82(3):233-238, 1974.

The pluricarcinogenic (syncarcinogenic) effects of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) on Swiss mice were studied. Random bred animals were treated i.p. with DMN (3 or 6 mg/kg) and/or DEN (3 or 6 mg/kg) once weekly for ten weeks. In comparison to untreated controls, the total lung tumor incidence was increased in the carcinogen-treated animals. Whereas DMN frequently caused lung carcinomas, DEN produced primarily lung adenomas. Atypical adenomas were generally found after simultaneous treatment with both carcinogens. Retroperitoneal vascular tumors and kidney adenomas were observed only after DMN treatment while forestomach papillomas were seen in animals treated with DEN or DEN plus DMN. Malignant lymphomas occurred in a lower incidence in the treated animals than in the controls, the lowest incidence being observed in the DMN and DMN plus DEN groups. The results indicate that neoplasms of the lymphopoietic system are not an appropriate parameter for evaluating the carcinogenic effects of DMN and DEN in Swiss mice. They also suggest a pluricarcinogenic effect of these agents in the Swiss mouse lung.

6119 AN ELECTRON MICROSCOPE STUDY OF REACTING ASTROCYTES IN GLIOMAS INDUCED BY N-ETHYL-N-NITROSOUREA IN RATS. *(E.)* Lantos, P. L. (Bland-Sutton Inst. Pathol., London, England). *Acta Neuropathol (Berl)* 30(2):175-181, 1974.

Reacting astrocytes in gliomas induced by *N*-ethyl-*N*-nitrosourea (ENU) were studied to determine their fine structure. The tumors were induced transplancentally by i.v. administration of 30 mg ENU/kg to BD-IX rats in the 15th day of gestation. In tumor-bearing animals astrocytes underwent striking morphological changes and showed increased mitotic activity, especially in white matter. Reacting astrocytes around gliomas displayed large, irregular nuclei with deep cytoplasmic invaginations, and the abundant cytoplasm contained in increased number of cell organelles. There were multiple Golgi complexes, many lysosomes and mitochondria, and an increased number of the wide cisternae of the rough-surfaced endoplasmic reticulum. The most dramatic increase is in glial filaments: these formed thick bundles at the periphery of the cells and in the processes which are frequently seen around demyelinated axons. Reacting astrocytes engulfed and broke down myelin sheaths: their remnants, lamellar inclusion bodies and lipid droplets, were present in the cytoplasm. Extravasated erythrocytes were also surrounded by and digested in reacting astrocytes. Numerous lysosomes could provide the necessary enzymes for the phagocytic activity of these cells. In contrast to neoplastic fibrillary astrocytes, reacting astrocytes were shown to have a larger number of more heterogeneous cytoplasmic inclusions. These results support the body of evidence that suggests that reacting astrocytes act phagocytically.

6120 CARCINOGENICITY OF *N*-METHYL-*N*-NITROSOUREA: POSSIBLE ROLE OF EXCISION REPAIR OF O^6-METHYLGUANINE FROM DNA. *(E.)* Kleihues, P. (Max-Planck-Inst. Hirnforsch, Cologne, West Germany) and G. P. Margison. *J Natl Cancer Inst* 53(6):1839-1841, 1974.

The persistence of the methylated purines produced *in vivo* by *N*-methyl-*N*-nitrosourea (MNU) in the DNA of rat tissues was studied. ^{3}H-MNU (10 or 80 mg/kg) was injected i.v. into young adult female BD-IX rats. After 6 or 78 hr, the animals were killed and DNA was prepared by phenol extraction from the liver, kidneys, and brain. The purine bases were separated from the purified DNA on Sehpadex G-10 columns. The average half-life of 3-methyladenine in the DNA of all three organs was 20 hr, while that of 7-methylguanine was 66 hr. In the brain, 67-79% of the O^6-methylguanine present at 6 hr remained at 78 hr, whereas in the kidney and liver, only 37-50% and 9-16%, remained after 78 hr. When the animals were treated with the low dose of MNU, O^6-methylguanine was removed from the liver DNA about nine times faster than from the brain DNA, the difference being only 4-fold following the higher dose of MNU. Tissue-specific differences in the persistence of O^6-methylguanine were even more marked when the amount of this base was expressed as a fraction of 7-methylguanine. The results suggest that the loss of O^6-aldylguanine from DNA may be mediated by an excision repair process which is dose dependent and which influences the carcinogenic action of alkylnitrosourea.

6121 ORIGINAL INVESTIGATIONS: CENTRAL NEUROBLASTOMA INDUCED BY TRANSPLACENTAL ADMINISTRATION OF METHYLNITROSOUREA IN WISTAR-R RATS. AN ELECTRON MICROSCOPIC STUDY.

(E.) Brucher, J. M. (Lab. Neuropathol., Univ. Louvain, Belgium) and A. E. Ermel. *J Neurol* 208(1):1-16, 1974.

A spinal cord tumor developing in a Wistar-R rat following the p.o. administration of methylnitrosourea (MNU) to its dam during gestation was examined by light and electron microscopy. Similar transplacental administration of MNU to other pregnant Wistar-R rats produced cerebral malformation in all offspring examined but did not produce other nervous system tumors. The one malignant neoplasm produced was diagnosed optically as a neuroblastoma. Although structures strongly suggestive of incipient or abortive synaptic formations were observed under the electron microscope, well-developed synapses were not observed. Among the various neuronal features displayed by the tumor cells, notably some extensive Nissl arrays and numerous subsurface cisternae, several were indicative of maturing nerve cells (branching tumoral processes with typical growth cones and dense-cored "neurosecretory" vesicles). The vesicles are considered to be one of the characteristics of newly developing nerve fibers. The presence of these developmental features in a nerve cell tumor helps to substantiate the embryonic origin of neuroblastomas which are postulated as arising from immature forms and not from dedifferentiated cells.

6122 FURTHER STUDIES ON INDUCTION OF STOMACH CANCER IN HAMSTERS BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE.

(E.) Kogure, N. (Natl. Cancer Cent. Res. Inst., Tokyo, Japan), H. Sasadairu, T. Kawachi, Y. Shimosato, A. Tokunaga, S. Fujimura and T. Sugimura. *Br J Cancer* 29(2):132-142, 1974.

To establish suitable conditions for the production of adenocarcinomas in the glandular stomachs of hamsters, the effect of a limited period of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) administration to male Golden hamsters was studied. Oral administration of MNNG at concentrations of 50-83 µg/ml in the drinking water resulted in a high incidence of tumors in the glandular stomach. Short-term administration of MNNG for 4-6 months resulted in a higher incidence of adenocarcinomas than did long-term administration for 7-8 months. One case of metastasis of an adenocarcinoma of the glandular stomach to the liver and two cases of metastasis to the regional lymph nodes were found and spindle cell sarcomas in the glandular stomach and adenocarcinomas in the duodenum were often produced. These findings were unrelated to the duration of treatment. Oral administration of MNNG at 500-2000 µg/ml for 4-7 days induced a hepatic cell carcinoma, intrahepatic bile duct carcinomas, bile duct cystadenomas and cystic dilatation and a hemangioma in the liver but no tumors in the glandular stomach. Sequential morphological studies on the glandular stomach tissue of hamsters receiving 50 µg/ml of MNNG in the drinking water showed three stages of change in the mucosa: the mucosa became atrophic and eroded during the first 16 weeks; irregular atypical glands developed at the margins of the erosions and proliferation of spindle cells in the submucosa were seen after 18 weeks and spindle cell sarcomas developed after 20 weeks. Adenocarcinomas developed between 25-32 weeks. The authors propose that the reason why hepatic tumors and cysts are formed on administration of higher concentrations of MNNG is due to its slow conversion to N-methyl-N'-nitroguanidine.

6123 CARCINOGENESIS OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE APPLIED INTO THE MOUSE BRAIN.

(E.) Tsunawaki, T. (Fac. Med., Kyushu Univ., Japan), M. Fukui, Y. Yamakawa and K. Kitamura. *Gann* 65(6):551-555, 1974.

The carcinogenic effects of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) applied locally to the mouse brain were studied. MNNG (0.2-0.3 mg) in crystal form was inserted into the cerebral hemispheres of male and female C3H and C57BL mice. Brain tumors were found in none of the 50 mice which survived longer than seven months, although a generally localized defect in the brain tissue was visible on the exposed surface. However, 18 of these mice developed tumors in other organs with the incidence of lung adenocarcinomas in male C57BL mice (3/12) and lymphomas in all mice (5/50) being particularly high. One mouse developed a squamous cell carcinoma in the scalp and a lymphoma in the neck. The results suggest that the brain of the adult mouse has little or no susceptibility to the carcinogenic action of MNNG but that this action can be transmitted to remote organs.

6124 PRODUCTION OF GASTRIC AND ESOPHAGEAL TUMORS IN RATS BY METHYLNITROSOCYANAMIDE, A POSSIBLE CANDIDATE OF ETIOLOGIC FACTORS FOR HUMAN GASTRIC CANCER.

(E.) Endo, H. (Fac. Med., Kyushu Univ., Fukuoka, Japan), K. Takahashi, N. Kinoshita and T. Baba. *Proc Jpn Acad* 50(7):497-502, 1974.

The carcinogenic effects of methylnitrosocyanamide (MNC), the mutagenic principle of nitrosated methylguanidine, in the stomachs of Sprague-Dawley rats were studied. MNC (1/10 the LD_{50} for mice) was administered in the drinking water to 20 female rats. After 11 months of continuous MNC treatment tumors were found in the forestomachs of the 11 surviving animals. With the exception of one squamous cell papilloma, all of the tumors were squamous cell carcinomas. One malignant tumor with a shallow ulcer and five small polyploid hyperplastic nodules with small foci of cystic dilatation and atypical cellular arrangements in the mucosa were seen in the glandular stomach. Three small squamous cell carcinomas and four small squamous cell papillomas were seen in the esophagus of seven rats. Among the animals which died before the 200th day of treatment, papillomas were observed in the forestomachs of two. The authors conclude that MNC is a potent gastric carcinogen in rats. Its potential carcinogen action in man remains to be determined.

6125 A RELATIONSHIP BETWEEN NUCLEAR DNA AND RNA SYNTHETIC ACTIVITIES AND THE CHANGES PRODUCED BY *N*-METHYL-*N*-NITROSO URETHANE: A STUDY USING *AMOEBA PROTEUS* AS A SINGLE CELL MODEL. *(E.)* Ord, M. J. (Dep. Biol., Univ., Southampton, England). *Chem Biol Interact* 8:269-283, 1974.

The degree of damage (inhibition of division, lethality, or inherited cellular changes) caused by *N*-methyl-*N*-nitroso urethane (MNU) during the cell cycle was investigated and fluctuation in cell sensitivity was related to changes in DNA and RNA synthetic activities (e.g. DNA configuration) at the time of treatment, using *Amoeba proteus* (strain P_{Da}, lines X67,69 and OW70) as a cell model. MNU treatment (1 x 10^{-3} M) of mixed age populations for short periods at 50 different times in their cell cycle produced wide differences in cell damage and led to the following conclusions: 1) although amoebae are sensitive to MNU at all ages, the dose needed to produce lethal damage to young and old cells varied by a factor of three, 2) cells were most sensitive at the time of division and during the peak of DNA synthesis, 3) smaller and temporary changes were found during the G_2 phase, some of which occur at times of intensive RNA synthesis, 4) the largest delay for first division occured in cells treated during division and S phase, while for second division it occurred in those treated during the mid to late G_2 phase, 5) cells treated during S phase had frequent phenotypic changes and during the S phase stable mutant lines were formed and 6) the length of survival of lethally-damaged cells (three to ten weeks), increased according to duration in the cell cycle at the time of treatment. Transfer of nuclei between treated and control cells proved that the cell sensitivity was dependent on changes in their nuclei. Supralethal doses (2-6 times whole cell dose) were required either to kill or cause a recognizable change in the offspring of viable cells. The relative insensitivity of older cells was determined not to be due to increased volume of their cytoplasm through experiments with cells having altered nuclear/cytoplasmic ratios.

6126 LACK OF AN EFFECT OF TUMOR-PROMOTING PHORBOL ESTERS AND OF G_1 CHALONE ON DNA SYNTHESIS IN THE EPIDERMIS OF NEWBORN MICE. *(E.)* Bertsch, S. (Dtsch. Krebsforsch. Inst. Biochem., Heidelberg, West Germany) and F. Marks. *Cancer Res* 34 (12):3283-3288, 1974.

The response of epidermal DNA synthesis to known tumor-promoting agents was compared in three age groups of NMRI mice. Phorbol esters 12-O-tetradecanoyl-phorbol-13-acetate (TPA), phorbol 12,13-didecanoate and TPA-20-^{3}H (specific activity 3.72 Ci/mM) were applied to the dorsal epidermis of newborn (10 µl), 1-week-old (40 µl) and adult females (100 µl). G_1 and G_2 chalones in aqueous extract of pigskin and extracts from mouse epidermis were injected i.p. at 5 µl/newborn, 10 µl/one week-old and 100 µl/adult. DNA was pulse labeled by i.p. injection of 1.5 µCi/g of thymidine-methyl-^{3}H (specific activity of 6.7 µCi/M) one hr before sacrifice. The DNA was then isolated and assayed for radioactivity. G_1 chalone caused strong inhibition of DNA synthesis in adults. In contrast, newborn mice exhibited a slight stimulation of thymidine incorporation (paradoxical chalone effect). Extracts from pure epidermis of newborn and adults gave similar results. DNA synthesis after one application of TPA was stimulated in adults (1,000%) and one-week-olds (100-200%) but not in newborns. In radioactivity studies there was no difference between newborns and adults. Keratinized skin of baby mice was removed by repeated stripping and phorbol didecanoate was applied with no result. However, increased epidermal thickness was observed 20 hr after TPA application in adults. The results show that susceptibility to phorbol esters increases with age. It is suggested that treatment with phorbol esters causes dedifferentiation of chalone-sensitive stem cells of the adult epidermis into chalone-insensitive stem cells similar to those in the fetus.

6127 SODIUM SACCHARIN: CYTOGENETIC EFFECT ON HUMAN LYMPHOCYTES *IN VITRO*. *(E.)* Chang, P. (Dep. Biol., California State Coll., Pa.) and T. Stacey. *Proc Pa Acad Sci* (48):50-51, 1974.

Chromatid aberrations were determined in human lymphocyte cultures incubated at 37 C with 0.002-2 mg/ml of sodium saccharin for 72 hr. Cultures containing low saccharin concentrations did not differ significantly from control cultures. At 2 mg/ml, saccharin produced chromosome gaps and 18 chromosome breaks/66 cells; the percentage of breaks (27.2%) differed significantly from the control. Lymphocytes treated with 20 mg/ml of saccharin appeared distorted and did not undergo mitosis. Tipolar and tetrapolar divisions were not observed. The single chromatid breaks produced by saccharin are important in terms of mutagenicity and carcinogenicity. Assuming a daily average saccharin concentration of 0.002 mg/ml, it may take a constant consumer 3-5 yr to reach the *in vivo* concentration (2 mg/ml) at which there would be mutagenic or carcinogenic damage. However, experimental data on body absorption and the excretion rate of saccharin are needed.

6128 THE INCIDENCE OF LUNG TUMORS IN C57BL MICE EXPOSED TO CIGARETTE SMOKE: AIR MIXTURES FOR PROLONGED PERIODS. *(E.)* Harris, R. J. C. (Imp. Cancer Res. Fund Lab., London, England), G. Negroni, S. Ludgate, C. R. Pick, F. C. Chesterman and B. J. Maidment. *Int J Cancer* 14(1):130-136, 1974.

The effects of air-dried and flue-cured tobacco smoke and the lung-tumor incidence in C57BL mice was studied. Young adult mice of both sexes were exposed for short periods at frequent intervals (approximately every other day) to smoke/air mixtures from cigarettes containing flue-cured and air-dried tobacco (both tobaccos from the same crop). Some of the mice were pretreated with influenza virus (given by aerosol) or antibiotics. The mice exposed to the smoke/air mixtures developed a higher incidence of lung tumors (adenomas and carcinomas), emphysema, and peribronchial and perivascular lymphocyte infiltration than did untreated controls. Smoke from the flue-cured tobacco was more toxic and induced cancer more quickly than that from air-dried tobacco, and the incidence of lung tumors was not affected by filtration of the smoke from the flue-cured tobacco. The mean age at death was significantly increased in both sexes by terramycin treatment. There was no synergistic effect between influenza virus infection and exposure to tobacco smoke. It was not clear whether exposure to the smoke/air mixtures shortened the lives of the mice.

6129 ASBESTOS BODIES AND FIBERS IN LUNG TISSUES. *(E.)* Fondimare, A. (Hosp. Calmette, Le Havre, France) and J. Desbordes. *Environ Health Perspect* 9:147-148, 1974.

6130 THE INGESTION OF ASBESTOS FIBERS. *(E.)* Webster, I. (Natl. Res. Inst. Occup. Dis., S. Afr. Med. Res. Counc., Johannesburg, South Africa). *Environ Health Perspect* 9:199-202, 1974.

6131 THE ULTRASTRUCTURAL EFFECTS OF AFLATOXIN B_1 IN THE RAT PANCREAS. *(E.)* Rao, M. S. (Univ. Kansas Med. Cent., Kansas City). *Fed Proc* 33(3):639, 1974.

6132 REACTIVITY OF H-2 AND TUMOR-SPECIFIC ANTIGENS ON METHYLCHOLANTHRENE-INDUCED TUMORS OF CONGENIC MICE. *(E.)* Klein, P. A. (Dep. Pathol., Univ. Florida, Gainesville). *Fed Proc* 33(3):791, 1974.

6133 DEMONSTRATION OF TUMOR-ASSOCIATED TRANSPLANTATION ANTIGEN(S) IN RAT MAMMARY TUMORS INDUCED BY 7,12-DIMETHYLBENZ(*a*) ANTHRACENE. *(E.)* Silinskas, K. C. (Dep. Biol., Univ. Windsor, Canada), W. Fang, A. B. Okey and T. K. S. Mukkur. *Fed Proc* 33(3):791, 1974.

6134 EFFECT OF SOME INHIBITORS OF ETHIONINE CARCINOGENESIS ON ETHIONINE METABOLISM. *(E.)* Brada, Z. (Papanicolaou Cancer Res. Inst., Miami, Fla.) and S. Bulba. *Fed Proc* 33(3):625, 1974.

6135 LIVER ULTRASTRUCTURE AND ZOXAZOLAMINE METABOLISM IN RATS TREATED WITH DL-ETHIONINE AND/OR PREGNENOLONE-16α-CARBONITRILE. *(E.)* Garg, B. D. (I.M.C.E., Univ. Montreal, Canada), B. Tuchweber and P. Kourounakis. *Fed Proc* 33(3):626, 1974.

6136 HISTOLOGICAL AND IMMUNOLOGICAL STUDIES OF COLON TUMORS IN LIPOTROPE-DEFICIENT RATS. *(E.)* Garmaise, A. B. (Massachusetts Inst. Technol., Cambridge, Mass.), C. A. Saravis and A. E. Rogers. *Fed Proc* 33(3):616, 1974.

6137 THE EFFECTS OF DIETHYLNITROSAMINE ON THE METABOLISM OF FOLIC ACID IN RATS. *(E.)* Shin, Y. S. (Univ. California, Berkeley), L. A. Poirier and E. L. R. Stokstad. *Fed Proc* 33(3):713, 1974.

6138 DIETARY EFFECTS ON INDUCED SLEEPING TIME AND AFLATOXIN RESPONSE IN RATS. *(E.)* Kula, N. S. (Massachusetts Inst. Technol., Cambridge, Mass.). *Fed Proc* 33(3):669, 1974.

6139 EFFECT OF THIOACETAMIDE (TA) ADMINISTRATION ON THE RAT LIVER NUCLEOLAR ACID SOLUBLE PROTEINS. *(E.)* Goldknopf, I. (Baylor Coll. Med., Houston, Tex.), N. R. Ballal, A. W. Prestayko, D. A. Goldberg and H. Busch. *Fed Proc* 33(3):635, 1974.

6140 FREE AND MEMBRANE-BOUND RIBOSOMES IN NORMAL AND METHYLCHOLANTHRENE TREATED MOUSE EPIDERMIS. *(E.)* Mueller, S. N. (Dep. Biol., Syracuse Univ., N.Y.) and T. S. Argyris. *Fed Proc* 33(3):603, 1974.

6141 EFFECTS OF ALTERED DIETARY FAT INTAKE ON SERUM PROLACTIN TITER. *(E.)* Cohen, L. A. (Am. Health Found., New York, N.Y.), F. Didato and P. C. Chan. *Fed Proc* 33(3):601, 1974.

6142 ALTERED INCORPORATION OF ^{3}H-THYMIDINE IN HAMSTER TRACHEAL EPITHELIUM CAUSED BY RESPIRATORY CARCINOGENS. *(E.)* Harris, C. C. (Natl. Cancer Inst., Bethesda, Md.), D. G. Kaufman, H. C. Boren and E. C. Wright. *Fed Proc* 33(3):601, 1974.

6143 HISTOPATHOLOGY OF RESPIRATORY SYSTEM OF BIO 15.16 HAMSTERS CHRONICALLY EXPOSED TO CIGARETTE SMOKE. *(E.)* Homburger, F. (Bio-Res. Consult., Cambridge, Mass.), P. Bernfeld and A. B. Russfield. *Fed Proc* 33(3):601, 1974.

6144 PERITONEAL MESOTHELIOMA. *(E.)* Kannerstein, M. (Mt. Sinai Sch. Med., New York, N.Y.) and J. Churg. *Fed Proc* 33(3):601, 1974.

6145 EFFECT OF HYPER(HA)- AND AVITAMINOSIS A(AA) ON URINARY BLADDER CARCINOGENICITY OF N-[4-(5-NITRO-2-FURYL)-2-THIAZOLYL]-FORMAMIDE(FANFT). *(E.)* Cohen, S. M. (St. Vincent Hosp., Worcester, Mass.), J. F. Wittenberg and G. T. Bryan. *Fed Proc* 33(3):602, 1974.

6146 DISPOSITION OF ALDRIN BY ISOLATED PERFUSED RABBIT LUNG PREPARATIONS. *(E.)* Mehendale, H. M. (Natl. Inst. Environ. Health Sci., Research Triangle Park, N.C.), E. A. El-Bassiouni and J. D. McKinney. *Fed Proc* 33(3):534, 1974.

6147 THE ENDOCRINE ROLE OF DIETARY LIPIDS IN MAMMARY TUMOR FORMATION. *(E.)* Gass, G. H. (South. Illinois Univ., Carbondale), W. T. Allaben, H. J. Brown, S. L. Liu and M. J. Bentley. *Fed Proc* 33(3):601, 1974.

6148 METABOLISM OF PYRIBENZAMINE AND ITS *N*-NITROSO DERIVATIVE IN THE RAT. *(E.)* Rao, G. S. (Natl. Inst. Health, Bethesda, Md.), G. Krishna and J. R. Gillette. *Fed Proc* 33(3):573, 1974.

6149 EFFECT OF CHLORDANE AND METYRAPONE PRETREATMENT ON THE BILIARY EXCRETION AND BINDING OF 2-ACETYLAMINOFLUORENE TO LIVER RIBOSOMAL-RNA IN RAT. *(E.)* Lee, O. S. (Coll. Pharm., Ohio State Univ., Columbus) and D. R. Feller. *Fed Proc* 33(3):574, 1974.

6150 STRUCTURE ACTIVITY RELATIONSHIP IN THE BINDING OF AFLATOXIN METABOLITES TO RAT LIVER RNA. *(E.)* Gurtoo, H. (Dep. Exp. Ther., Roswell Park Mem. Inst., Buffalo, N.Y.) and C. Dave. *Fed Proc* 33(3):574, 1974.

6151 THE LOCALIZED EFFECT OF DEPOT INJECTIONS OF VITAMIN A ON NORMAL RAT SKIN AND SKIN TREATED WITH 7,12-DIMETHYLBENZANTHRACENE. *(E.)* Brown, I. V. (Schering Corp., Bloomfield, N.J.). *Fed Proc* 33(3):574, 1974.

6152 THE APPLICATION OF CHEMICAL CARCINOGENS TO PLANTS. *(E.)* Kaiser, H. E. (No affiliation). *Fed Proc* 33(3):595, 1974.

6153 MALONDIALDEHYDE INCREASES DURING CARCINOGEN INDUCED CHROMOSOME DAMAGE. *(E.)* Shamberger, R. J. (Cleveland Clin. Found., Ohio), S. A. Tytko and C. E. Willis. *Fed Proc* 33(3):595, 1974.

6154 CHROMOSOME DAMAGE AND EXPERIMENTAL POLYCYCLIC HYDROCARBON CARCINOGENESIS. *(E.)* Wolfe, R. N. (Univ. Kentucky Med. Cent., Lexington, Ky.), J. Q. Lowry, A. E. Shuck and E. D. Rees. *Fed Proc* 33(3):595, 1974.

6155 SEQUENTIAL BIOCHEMICAL CHANGES IN HYPERPLASTIC AREAS IN THE LIVER OF RATS FED DIETHYLNITROSAMINE (DEN). *(E.)* Kitagawa, T. (McArdle Lab., Univ. Wisconsin, Madison) and H. C. Pitot. *Fed Proc* 33(3):595, 1974.

6156 MORPHOLOGICAL TRANSFORMATION OF ORGAN CULTURED EMBRYONIC RAT LIVER BY N-2-FLUORENYLACETAMIDE (2-FAA). *(E.)* Parsa, I. (Downstate Med. Cent., State Univ. New York, Brooklyn). *Fed Proc* 33(3):595, 1974.

6157 EFFECT OF DIETHYLDITHIOCARBAMATE (DEDTC) AND AMINOACETONITRILE (AAN) ON THE INDUCTION OF STRAND BREAKS IN RAT LIVER DNA BY CHEMICAL CARCINOGENS *IN VIVO*. *(E.)* Sarma, D. S. R. (Temple Univ. Sch. Med., Philadelphia, Pa.), S. Rajalakshmi and D. Hadjiolov. *Fed Proc* 33(3):595, 1974.

6158 ASSOCIATION OF INCREASED MUTAGENESIS RATE *IN VITRO* WITH THE GENETICALLY MEDIATED INCREASE IN MOUSE LIVER MICROSOMAL ARYL HYDROCARBON HYDROXYLASE ACTIVITY *IN VIVO*. *(E.)* Felton, J. S. (Natl. Inst. Child.Health Dev., Bethesda, Md.) and D. W. Nebert. *Fed Proc* 33(3):596, 1974.

6159 TUMOR DEVELOPMENT IN RATS CAUSED BY ENDOGENOUS CARCINOGENS PRODUCED AFTER ADMINISTRATION OF *n*-PROPYL-, *iso*-PROPYL- AND *n*-BUTYLUREA AND NITRITE. *(Ger.)* Schneider, J. (Med. Acad., Erfurt, E. Germany), U. Bicker, R. Warzok and G. Osske. *Arch Geschwulstforsch* 44(2):126-133, 1974.

6160 BENZENE-INDUCED LEUKEMIA. *(Ger.)* Stieglitz, R. (1st Med. Clin., Humboldt Univ, Berlin, E. Germany), H. Stobbe and W. Schuttman. *Arch Geschwulstforsch* 44(2):145-148, 1974.

6161 EXPERIMENTAL OSTEOSARCOMAS INDUCED BY BERYLLIUM. *(Ger.)* Komitowski, D. (German Cancer Res. Cent., Heidelberg, W. Germany). *Verh Dtsch Ges Pathol* 58:438-440, 1974.

6162 HEPATOCELLULAR GLYCOGEN STORAGE AND THE ETIOLOGY OF HYPERPLASTIC LIVER NODULES IN RATS WITH THIOACETAMIDE INTOXICATION. *(Ger.)* Bannasch, P. (Pathol. Inst., Univ. Wurzburg, W. Germany), J. Hesse and H. Angerer. *Virchows Arch [Zellpathol]* 17(1):29-50, 1974.

6163 THE POSSIBLE CARCINOGENICITY OF OVULATION INHIBITORS STUDIED BY CYTOPHOTOMETRY AND WITH THE INTERFERENCE MICROSCOPE. *(Ger.)* Herzog, R. E. (Clin. Obstet. Gynecol., Univ. Mainz, W. Germany). *Arch Gynaekol* 217(4):443-454, 1974.

6164 EARLY TISSUE CHANGES IN SOME VARIANTS OF CHEMICAL CARCINOGENESIS. *(Rus.)* Avtsyn, A. P. (Inst. Human Morphol., Moscow, USSR) and L. Ia. Iablonovskaia. *Arkh Patol* 36(8):46-54, 1974.

6165 SOME INTRINSIC AND EXTRINSIC ASPECTS OF HYDRAZINE CARCINOGENESIS. *(It.)* Biancifiori, C. (Dept. Oncol. Res., Univ. Perugia, Italy). *Tumori* 60(5):429-435, 1974.

See also:

* (Rev): 6001, 6002, 6003, 6004, 6005, 6006, 6007, 6008, 6009, 6010, 6011, 6012, 6013, 6046, 6055, 6057, 6058, 6061, 6064, 6067, 6068, 6072, 6074, 6078, 6079, 6083
* (Phys): 6175
* (Viral): 6178, 6182, 6218
* (Immun): 6266, 6269, 6353, 6356, 6357
* (Epid-Biom): 6421, 6422, 6423, 6447, 6448

6166 CHROMOSOME LESION MORPHOLOGY AND CHANGES IN EHRLICH ASCITIC TUMOR CELL MARKER CHROMOSOMES AFTER IRRADIATION.

(It.) Giriodi, P. F. (Holy Cross Hosp., Cuneo, Italy). *Arch Sci Med (Torino)* 131(2):74-82, 1974.

Karyotyping procedures and morphology of chromosome lesions are reviewed and the effects of radiation on chromosome aberration are reported with respect to the average size of irradiated marker chromosomes. Using Ehrlich ascitic tumor cells (twin strain), examination of metaphasic plates at 2 hr intervals over a 24 hr period after irradiation (300 R) showed that figures on chromosome, chromatid and subchromatid aberrations were expressions of the different points of the vital cycle in which cells were irradiated. A further investigation to verify other chromosomal lesions by statistical analysis of marker chromosome dimensions showed no statistically significant change in the size of the marker chromosomes.

6167 INFLUENCE OF X-IRRADIATION ON THE DIFFERENT STAGES OF THE CELL CYCLE IN REGENERATING RAT LIVER.

(E.) Barbason, H. (Univ. Liege, Lab. Anat. Pathol., France). *Arch Otorhinolaryngol (NY)* 206(4):337-343, 1974.

The influence of X-irradiation on the different stages of the cell cycle in regenerating rat liver was investigated using five groups of Wistar rats which were partially hepatectomized. The first group was the non-irradiated control and the others were irradiated (450 R) at different post-operative delays of 1 hr (hepatocytes in G_0), 12 hr (middle of G_1), 20 hr (end of G_1--beginning of S) and 28 hr (S phase). When the irradiation was performed in G_0 or G_1, the same inhibition of mitotic waves, micronuclei and anatelophasic bridges index was observed. However, the mitotic delay induced by the irradiation in G_0 was completely reduced when the rats were irradiated after the middle of G_1 (from the 12th post operative hr). When the irradiation was performed in the S phase, the first mitotic wave was almost completely inhibited. This suggests that a certain number of hepatocytes start to divide but remain blocked before the anatelophase and become pycnotic. On the contrary, the second mitotic wave was the same as in the other irradiated groups and the micronuclei and the bridges appeared at its level. This suggests that hepatocytes irradiated in S phase remain blocked in pre-mitosis up the second nyctohemeral period favorable to their division. The results indicate that each phase of the cell cycle corresponds to a specific radiosensitivity that the irradiation may induce abnormalities which differ according to these phases.

6168 THE INCIDENCE OF LUNG METASTASES IN C3H MICE AFTER TREATMENT OF IMPLANTED SOLID TUMOURS WITH X-RAYS OR SURGERY.

(E.) Sheldon, P. J. (Mt. Vernon Hosp., Northwood, England), A. C. Begg, J. F. Fowler and I. F. Lansley. *Br J Cancer* 30(4):342-348, 1974.

The incidence of lung metastases in C3H mice was investigated after the mice were implanted with pieces of spontaneous mammary carcinoma which were irradiated or removed surgically when they had grown to 6.5 mm mean diameter. The incidence of lung metastases was determined from samples taken at various times up to six months later. Single X-ray doses and fractionated schedules (up to 15 fractions in 18 days) were used. No significant differences were observed in the results for all these schedules. There was a substantially higher incidence in mice in which the treatment failed than in those cured and there was the same incidence of metastases whether the mice were cured by surgery or by radiation. The incidence of metastases was 8% if the implanted tumor was cured by the radiation, whereas if the radiotherapy did not cure the tumors, the incidence was 35%. This difference between the two groups was significant. If tumors recurred locally after radiotherapy and were then removed surgically, the incidence of lung metastases was significantly greater than that after surgery of non-irradiated tumors. The incidence of metastases was similar after curative surgery and after curative radiotherapy. The data did not provide definitive evidence that local non-curative irradiation increases the likelihood of a mouse developing lung metastases.

6169 NASCENT DNA SYNTHESIS IN ULTRAVIOLET LIGHT-IRRADIATED MOUSE, HUMAN, AND CHINESE HAMSTER CELLS.

(E.) Rauth, A. M. (Dep. Med. Biophys., Univ. Toronto, Canada), M. Tammemagi and G. Hunter. *Biophys J* 14(3):209-220, 1974.

The ability of three different established cell lines to synthesize DNA after various exposures to UV irradiation was investigated in mouse L, human HeLa and Chinese hamster ovary cells using the technique of alkaline sucrose gradient centrifugation. The cells were exposed to 0, 100, 200, or 400 ergs/mm^2 of UV light in medium deficient in thymidine. The DNA profiles for all three cell lines at 0 exposure were similar with peaks occurring at fractions 14-16. However, at short times after UV light irradiation, human HeLa cells appeared to synthesize more low molecular wt DNA than either mouse L or Chinese hamster ovary cells. After 100 ergs/mm^2 the molecular weight distribution for HeLa cells shifted much more strongly to the low molecular weight region than for L cells. As the irradiation exposure increased, the differences became less, and at 400 ergs/mm^2 both L and HeLa cell nascent DNA shifted to low molecular weight. Chinese hamster ovary cells appear intermediate between L and HeLa cells in the effect of UV irradiation on the molecular weight distribution of newly synthesized DNA. Since these differences are not related to differences in either the rate of DNA synthesis or amount of UV damage in the irradiated cells, it appears to be a phenotypic characteristic of the cell lines tested. In addition, there seems to be a correlation for L, HeLa and Chinese hamster ovary cells between the size of the segments of DNA synthesized after UV irradiation and the survival of the cells as measured by their colony forming ability.

6170 MODIFICATION OF CELL CYCLE RESPONSE OF SYNCHRONOUS MAMMALIAN CELLS TO IONIZING RADIATION BY INHIBITION OF REPAIR. *(E.)* Sinclair, W. K. (Div. Biol. Med. Res. Argonne Natl. Lab., Ill.). *Cold Spring Harbor Conf Cell Proliferation* 1:985-994, 1974.

Factors that appear to control the lethal radiation response and its fluctuations during the cell generation cycle were determined using the V79 line of Chinese hamster lung cells. The cells were synchronized by mitotic selection and labeled with ^{3}H-thymidine. Maximum survival did not occur at the maximum rate of DNA synthesis (middle of S) or when all of the DNA was synthesized (end of S), but in between, about two thirds of the way through S. When cells were prevented from synthesizing DNA, survival first fell sharply and then rose again late in the cell cycle. These facts suggest that another factor (named Q), which varies as a function of cell cycle position, also controls cell survival. Investigations of the identity of Q suggest that it may be some small fraction of the intracellular sulfhydryl. Evidence in support of this was provided by the fact that the aminothiol cysteamine protects cells from lethal damage caused by X-irradiation differentially during the cell cycle, the most sensitive cells being protected the most. The sulfhydryl binding agent *N*-ethylmaleimide sensitizes cells differentially during the cell cycle and suppresses the rise in survival late in the cell cycle of hydroxyurea-inhibited cells. The capability to repair damage appears to be dependent both on DNA synthesis and on the presence of some substances containing sulfhydryl. Thus hydroxyurea and *N*-ethylmaleimide together can strip the cell of all capacity to repair radiation damage, but neither one alone can do it completely. This generalization may only be true to a first approximation, but it may account for a large portion of the variation in response expressed as lethal damage during the cell cycle.

6171 KIDNEY AND BONE RETENTION FUNCTIONS IN THE HUMAN METABOLISM OF URANIUM. *(E.)* Adams, N. (Natl. Radiol. Prot. Board, Harwell, England) and N. L. Spoor. *Phys Med Biol* 19(4):460-471, 1974.

The retention of uranium by bone and kidney was reevaluated taking into account recently published data for a man who had been occupationally exposed to natural uranium aerosols for 10 yr and for adults who had ingested uranium at the normal dietary levels of about 1 g/day. For lifetime occupational exposure to uranium aerosols the new retention functions yield a greater retention in bone and a smaller retention in kidney than the earlier ones, which were based on acute intakes of uranium by terminal patients. Hence, bone replaces kidney as the critical organ. The new evidence points to the conclusion that the current $(MCP)_a$ for ^{238}U (6.6 x 10^{-11} μCi cm^{-3}) which is based on chemical toxicity is more restrictive than any value based on radiological assessment.

The same evidence may be used, within the framework of the 1959 lung model, to support an $(MCP)_a$ for natural uranium (8.4 X 10^{-11} μCi cm^{-3}) based on radiological considerations which are more restrictive than that currently recommended by the ICRP and ACGIH and based on chemical toxicity. This more restrictive $(MCP)_a$ corresponds to a mass concentration of 126 gm^{-3}, a level which has long been used as a working limit in some parts of the nuclear industry.

6172 BONE TUMOR RISK IN MICE AFTER SINGLE AND REPEATED INJECTIONS OF ^{224}Ra. *(E.)* Gossner, W. (X-Ray, Environmental Res. Soc., Nuremberg, West Germany), A. Luz, W. A. Muller and O. Hug. *Radiat Res* 59(1):55, 1974.

6173 MECHANISM OF RADIATION CARCINOGENESIS. *(E.)* van Bekkum, D. W. (Radiobiol. Inst., TNO, Rijswijk, Netherlands). *Radiat Res* 59(1):8, 1974.

6174 THYROID TUMORS OCCURRING AS LATE EFFECTS OF HEAD AND NECK IRRADIATION: PRELIMINARY REPORT. *(E.)* Frohman, L. (Michael Reese Med. Ctr., Chicago, Ill.), J. Arnold, M. Colman, M. Favus, S. Pinsky, Y. Ryo, A. Schneider and M. Stachura. *Clin Res* 22(4):599A, 1974.

6175 POLONIUM-210 ALPHA RADIATION AS A CANCER INITIATOR IN TOBACCO SMOKE. *(E.)* Radford, E. P. (Johns Hopkins Univ., Sch. Hyg., Public Health, Baltimore, Md.). *Radiat Res* 59(1):223, 1974.

6176 ULTRASTRUCTURE OF RADIATION-INDUCED OSTEOSARCOMAS IN MICE. *(Ger.)* Marquart, K. H. (Soc. Radiat. Environ. Res., Munich, West Germany), A. Luz and W. Gossner. *Verh Dtsch Ges Pathol* 58: 434-437, 1974.

6177 THE IRRADIATION OF FOOD PRODUCTS. *(Fr.)* Bugyaki, L. (Inst. Hyg. Epidemiol., Brussels, Belgium). *J Belge Radiol* 57(5):387-407, 1974.

See also:

* (Rev): 6072, 6077
* (Chem): 6085
* (Viral): 6252
* (Immun): 6298, 6353

6178 RNA TUMOR VIRUS GENOMES AS DETERMINANTS OF CHEMICALLY-INDUCED TRANSFORMATION *IN VITRO*. *(E.)* Freeman, A. E. (Microbiol. Assoc., Inc., Bethesda, Md.), P. J. Price, E. M. Zimmerman, G. J. Kelloff and R. J. Huebner. *Bibl Haematol* (39):617-634, 1973.

Studies of the individual and combined effects of chemicals and leukemia viruses as inducers of cell transformation *in vitro* are reported. In rat embryo cultures (Fischer, Osborne-Mendal, Wistar), transformation occurred only in cultures treated with a combination of a murine leukemia virus and a chemical carcinogen (diethylnitrosamine, 3-methylcholanthrene, benzo(a)pyrene, city smog residues, or cigarette smoke condensates). Transformation could not be demonstrated in cultures treated with either virus or chemicals as individual agents. Diethylnitrosamine-transformed cultures did not induce tumors in isogenic newborn rats inoculated s.c. with 1-2 x 10^6 cells, but tumors were obtained with cultures transformed by the other chemicals. The *in vivo* carcinogenic activity of a series of azo dyes, aromatic amines, and hydrocarbons was studied in relation to the following characteristics of *in vitro* transforming activity: time required for ' initial transformation, number of foci, optimal dose, and ability to transplant tumors in animals. In general, compounds that were not carcinogenic *in vivo* were inactive *in vitro*. However, some chemicals (e.g., 3,3'-dichlorobenzidine) that were weakly active *in vivo* were strong transforming agents *in vitro*. To determine whether C-type RNA viruses are implicated in cellular transformation by chemical carcinogens, transformation studies were repeated with hamster embryo cells. Hamster leukemia virus (HaLV)-infected cultures were ten times more sensitive to transformation by extracts of city smog residue than HaLV-free cultures; however, virus-free cells were readily transformed by the chemical. Syrian hamster embryo cultures transformed by 3-methylcholanthrene and cigarette smoke residue produced tumors in newborn hamsters after inoculation of 2 x 10^6 cells. Six of nine cell lines derived from the tumors were positive for HaLV antigen compared with none of the original or transformed lines. HaLV was also found in cell lines derived from tumors induced *in vivo* by chemical carcinogens, but not in lines derived from SV40-induced tumors. Thus, isolation of virus was correlated with chemical treatment and not merely with tumor induction. Rauscher leukemia-infected rat cell lines often contained up to 10% of transformed colonies. It is concluded that chemicals accelerate the rate of transformation by blocking or derepressing the host cell gene control mechanisms. It is also postulated no cell culture is completely free of RNA tumor virus genetic information.

6179 BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SURFACE CHANGES IN NORMAL, MSV- AND SV40-TRANSFORMED, AND SPONTANEOUSLY TRANSFORMED CLONES OF BALB/C CELLS. *(E.)* Grimes, W. J. (Coll. Med., Univ. Arizona, Tucson). *Cold Spring Harbor Conf Cell Proliferation* 1:517-531, 1974.

Primary and secondary normal cells and their spontaneous and viral transformants were studied to determine the involvement of increased invasiveness, altered immunogenicity, and loss of contact inhibition in cancer. Cultures of primary and secondary Balb/c mouse fibroblasts and Simian virus (SV40)-, murine sarcoma virus (MSV)-, and spontaneously-transformed Balb/c derivatives were compared with respect to carbohydrate conposition, sialic acid transferase level, ability to transport 2-deoxyglucose, agglutination, contact inhibition, and tumorigenicity. The results indicated that increased transport of (^3H)2-deoxyglucose was not a necessary prerequisite for failure of contact inhibition in transformed cells. The SV40 and spontaneous transformants showed higher than normal levels of agglutination, the MSV transformants being less agglutinable than the other transformed lines; the time in culture had little effect on agglutination. The SV40- and spontaneously-transformed cells produced tumors in immunocompetent Balb/c mice; these tumors regressed more or less rapidly. The MSV-transformed cells were highly tumorigenic, inducing tumors which showed no evidence of regression. The normal cells produced no tumors. The spontaneous transformants appeared to require increased natural agglutinability for tumorigenicity. The levels of sialic acid and sialic acid transferase were reduced in the SV40-transformed cells and several clones derived from the normal cells, and the carbohydrate levels in all of the transformed lines were reduced relative to the normal cells. These results suggest that several different pathways may lead to cell transformation.

6180 VIRAL DNA IN TRANSFORMED CELLS. II. A STUDY OF THE SEQUENCES OF ADENOVIRUS 2 DNA IN NINE LINES OF TRANSFORMED RAT CELLS USING SPECIFIC FRAGMENTS OF THE VIRAL GENOME. *(E.)* Gallimore, P. H. (Med. Sch., Birmingham, England), P. A. Sharp and J. Sambrook. *J Mol Biol* 89(1):49-72, 1974.

In studying the sequences of DNA from adenovirus 2, ^{32}P-labeled adenovirus 2 DNA was treated with restricting endonuclease from *Hemophilus parainfluenzae* (*Hpa* I) or restricting endonuclease from *Escherichia coli* strain RY-13 (*Eco*RI). The resulting DNA fragments were separated by means of gel electrophoresis. Measurements were made of the kinetics of renaturation of each of the fragments and also of complete adenovirus 2 DNA. This was done in the presence of DNA, which was extracted from nine lines of rat cells (adenovirus 2-transformed). Of these transformed cell lines, six contained sequences of viral DNA homologous to part of one of the six *Eco*RI fragments and to two of the seven *Hpa* I fragments. The cell lines only contain the viral DNA segment stretching from the left end until a point that is 14% along the viral genome. Any viral function that is expressed in the transformed cells has to be coded by this section of the viral DNA.

6181 ABSENCE OF ADENOVIRUS-SPECIFIC REPRESSOR IN ADENOVIRUS TUMOUR CELLS. *(E.)* Weber, J. (Dep. Microbiol., Cent. Hosp. Univ., Sherbrooke, Canada). *J Gen Virol* 22(2):259-264, 1974.

The possible presence of a repressor-like mechanism responsible for the maintenance of the viral genome in integrated form in the transformed cell was studied using adenovirus type 12 (Ad 12) tumor cells (HTC). Sendai virus mediated fusion of HTC and permissive cells (HEp-2) was used to rescue Ad 12 from the former. No evidence of virus rescue was obtained. Heterokaryocytes of HTC and HEp-2 cells were then superinfected with Ad 12 to test for the presence of repressor in the transformed cells. At the highest multiplicity of infection, all of the nuclei of both types of cells generally contained inclusion bodies, the ratio of inclusion bodies in the cells being independent of the relative numbers of the two types of nuclei. Furthermore, both types of heterokaryocytes were represented among the heterokaryocytes. The synthesis of virus antigens by the heterokaryocytes was examined by immunofluorescence. The results indicated that following infection of the heterokaryocyte, the viral proteins were transported to all the nuclei within the heterokaryocyte. The data argue against the presence of a repressor or repressor-like mechanism in the Ad 12 tumor cell.

6182 ENHANCEMENT OF VIRAL ONCOGENESIS BY CHEMICAL CARCINOGENS. *(E.)* Castro, B. C. (BioLabs., Inc., Northbrook, Ill.). *Biochem Dis* 4 (Part B):607-618, 1974.

Hamster embryo cells (HEC) treated with chemical carcinogens are more susceptible to transformation by an oncogenic simian adenovirus, SA7. Pretreatment (18 hr before virus inoculation) of HEC with 3-methylcholanthrene (MC), benzo(a)pyrene (BP), dibenz(a,h)anthracene, dibenz(a,c)anthracene, and 7, 12-dimethylbenz(a)anthracene (DMBA) increased the frequency of SA7 transformation; pretreatment with phenanthrene, perylene, or pyrene did not. Concentrations of MC, BP, and DMBA above those necessary to demonstrate maximal enhancement often reduced the frequency of transformation relative to that observed with low doses. Addition of these three compounds 5 hr after virus inoculation resulted in a dramatic inhibition of virus transformation. Pretreatment of HEC (2 or 18 hr) with methyl methanesulfonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), or acetoxy-N-2-acetylaminofluorene (ac-AAF) also enhanced SA7 transformation; methylazomethanol acetate stimulated transformation when added 18 hr, but not 2 hr, before virus inoculation. Enhancement of viral transformation was also demonstrated when MMS, ac-AAF, or MNG was added 5 hr after virus inoculation and cell transfer. N-nitrosodiethylamine, N-nitrosodimethylamine, and N-2-acetylaminofluorene did not stimulate viral transformation in HEC. It is suggested that the role of chemical carcinogens in promoting viral transformation may be due to the creation of gaps in cell DNA, thereby providing additional sites of incorporation of viral genetic material into cell DNA.

6183 SYNTHESIS AND ASSEMBLY OF ADENOVIRUS TYPE 2 POLYPEPTIDES. II. REVERSIBLE INHIBITION OF HEXON ASSEMBLY AT 42^0. *(E.)* Leibowitz, J. (Albert Einstein Coll. Med., Bronx, N.Y.) and M. S. Horwitz. *Virology* 61(1):129-139, 1974.

Adenovirus type 2-infected KB and HeLa S_3 cells were radiolabeled (with ^{14}C-amino acids) and fractionated, and the total viral peptides and assembled hexon and penton capsomeres determined by SDS-polyacrylamide gel electrophoresis. Compared with the level seen at 37 C, there was a 40% block in peptide assembly in cells incubated at 42 C. A more striking defect was noted in the assembly of the viral capsomeres from the proteins synthesized at 42 C; at this temperature, only 3% of the monomeric hexon polypeptides assembled to form the trimeric capsomere. A slightly less marked block occurred in the assembly of the penton capsomere from the penton base and fiber polypeptides. The block in capsomere assembly at the elevated temperature was reversible after temperature stepdown to 37 C. Hexon assembly was not dependent on ongoing protein synthesis, although the kinetics of assembly after stepdown were somewhat slower in the absence of new protein synthesis. The molecular weights of the hexon polypeptides synthesized at 37 C and 42 C were equal. The removal of the N-terminal methionine from hexon appeared to take place normally at 42 C, and the phenylalanine-containing tryptic peptides were identical at both temperatures. Of a variety of metabolic inhibitors tested, only cyanide and mercaptoethanol reduced the amount of hexon assembled after stepdown to 37 C. There appeared to be no intrachain disulfides in freshly isolated hexon polypeptides at either temperature. The data suggest that factors other than the primary structure of the polypeptides are important for their *in vivo* assembly.

6184 VISUALIZATION OF THE INVERTED TERMINAL REPETITION IN ADENO-ASSOCIATED VIRUS DNA. *(E.)* Berns, K. I. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.) and T. J. Kelly, Jr. *J Mol Biol* 28(1):267-272, 1974.

Due to their probable significance in DNA replication, the termini of adeno-associated virus DNA were characterized. Adeno-associated virus 2, containing ^{14}C-labeled DNA in which bromodeoxyuridine had been partially substituted for thymidine (40%), was grown in KB cells in spinner culture. The heavy single strains of bromodeoxyuridine-labeled virus DNA were isolated and annealed at a concentration of 20 μg/ml in 50% formamide at room temperature for 24 hr. Representative single-stranded circular monomers and dimers of the virus DNA with projections were observed by electron microscopy. Of 107 single-stranded monomers photographed, 82 had only one projection and seven had no visible projection. Of 25 single-stranded circular dimers photographed, 16 had two projections, and the remainder, three or four. Measurements of 71 circular monomers and 25 circular dimers yielded average lengths of 1.56 μm and 3.12 μm, resp. The relative interprojection distances measured for dimers with only two projections were plotted: the majority of projections (48/61) occurred in the relative positions expected along the length of single-stranded circular dimers, and most likely represent the hydrogen-bonded, inverted terminal repetition regions holding these dimers together. The relative lengths of projections were determined for 50 single-stranded circular monomers with one projection. The results were compatible with the possibility that this DNA contains a terminal repetition whose sequence is symmetrical (122'1 - - - - 122'1'). In conclusion, these observations are compatible with an inverted terminal sequence of the type 12 - - - - 2'1', which would allow base pairing of complementary regions in the anti-parallel configuration.

6185 EVIDENCE FOR CROSSING-OVER BETWEEN AVIAN TUMOR VIRUSES BASED ON ANALYSIS OF VIRAL RNAS.

(E.) Beemon, K. (Dep. Mol. Biol., Univ. California, Berkeley), P. Duesberg and P. Vogt. *Proc Natl Acad Sci USA* 71(10):4254-4258, 1974.

The RNAs of several avian tumor virus recombinants which had inherited their focus-forming ability from a sarcoma virus and their host range marker from a leukosis virus were investigated. Electrophoretic analyses showed that the cloned sarcoma virus recombinants contained only size class *a* RNA, although they had acquired a marker which resided on class *b* RNA in the leukosis virus parent. Class *a* RNA of different recombinant clones, derived from the same pair of parental viruses and selected for the same biological markers, differed slightly from each other and from the parental sarcoma virus in electrophoretic mobility. They also had different fingerprint RNase T1-resistant oligonucleotides. The average complexity of the 60-70S RNA prepared from the Prague Rous sarcoma virus of subgroup B was estimated to be 3.5 X 10^6 daltons; the estimate was based on the size of 20 RNase T1-resistant oligonucleotides, which represented 3.9% of the RNA. Based on 23 oligonucleotides which represented 4.7% of the RNA, the average complexity of the 60-70S prepared from a recombinant was estimated at 3.3 X 10^6 daltons. Thus, the genome of the wild-type and recombinant RNA tumor viruses appeared to be polyploid. The data suggest that recombination among the avian tumor viruses occurred by crossing-over between homologous pieces of nucleic acid.

6186 THE NUCLEOTIDE SEQUENCE COMPLEXITY OF AVIAN TUMOR VIRUS RNA.

(E.) Billeter, M. A. (Inst. Mol. Biol., Univ. Zurich, Switzerland), J. T. Parsons and J. M. Coffin. *Proc Natl Acad Sci USA* 71(9):3560-3564, 1974.

The nucleotide sequence complexity of 60-70S RNA of Rous sarcoma virus was determined from the molar yields of 11 pure oligonucleotides of known chain length, obtained from [^{32}P]RNA of Rous sarcoma virus by T1 RNase digestion and two-dimensional polyacrylamide gel electrophoresis. An apparent chain length of 9800 ± 2500 nucleotides was calculated, corresponding to a molecular weight of 3.4 $\pm$ 0.86 x 10^6. Assuming the molecular weight of 60-70S RNA of Rous sarcoma virus to be about 10^7, the data suggest that the virus genome consists of identical or very similar, nucleotide sequences repeated three to four times.

6187 SUPPRESSION OF THE AVIAN SARCOMA VIRUS GENOME IN HAMSTER-TRANSFORMED CELLS MADE RESISTANT TO 8-AZAGUANINE.

(E.) Hladka, M. (Cancer Res. Inst., Slovak Acad. Sci., Bratislava, Czechoslovakia) and C. Altaner. *J Natl Cancer Inst* 53(5): 1221-1227, 1974.

Properties of hamster cells [Ha(SR)] transformed by the Schmidt-Ruppin strain of avian sarcoma virus and made resistant to 8-azaguanine (aza-G) were studied. The uncloned resistant cell line [Ha(SR)-AG-50] proliferated in the presence of 50 µg aza-G/ml in medium supplemented with hypoxanthine, amethopterin, thymidine, and glycine (HATG) and in normal medium. To determine whether aza-G-resistant cells were heterogenous with respect to rescuable RSV genome, tumorigenicity, and enzyme deficiency, 16 single-cell clones were isolated from Ha(SR)AG-50 cells in medium containing 50 µg aza-G/ml. All clones were resistant to aza-G. Two of 16 clones were deficient in hypoxanthine-quanine phosphoriboysl transferase (HGPRT) activity; they did not grow in HATG, did not incorporate labeled hypoxanthine, and had negligible HGPRT activity. Resistance of the clones to aza-G was genetically stable. The aza-G-resistant cells were less tumorigenic than the parental cell line which induced progressive tumors in adult hamsters at a dose of 10^3 cells. Several cell clones were extensively tested for virus genome by fusion with chicken embryo cells and subsequent inoculation of cells to chickens. The virus genome was strongly suppressed in all aza-G-resistant clones, despite prolonged cultivation of resistant cells in normal medium. The suppression was not connected with HGPRT deficiency. Avian group-specific antigen was inhibited in all aza-G-resistant clones compared with the parental clonal cell line. Results suggest that the presence or absence of virus genome in already transformed cells is not strictly correlated with the tumorigenicity of the cells. There is also no direct relationship between transformed phenotype *in vitro* and the ability of the cell to form tumors *in vivo*.

6188 SYNTHESIS OF VIRAL DNA IN THE CYTOPLASM OF DUCK EMBRYO FIBROBLASTS AND IN ENUCLEATED CELLS AFTER INFECTION BY AVIAN SARCOMA VIRUS.

(E.) Varmus, H. E. (Dep. Microbiol., Univ. California, San Francisco), R. V. Guntaka, W. J. W. Fan, S. Heasley and J. M. Bishop. *Proc Natl Acad Sci USA* 71(10): 3874-3878, 1974.

The intracellular site of viral DNA synthesis in avian sarcoma virus (ASV)-infected duck embryo fibroblasts and enucleated duck embryo fibroblasts was studied by direct measurement in cytoplasmic extracts. All of the viral DNA was confined in the cellular cytoplasm three hr after viral infection and over 80% remained in the cytoplasm after six hr. Between six and nine hr after infection, however, the viral DNA appeared to be transported to the nucleus. The viral DNA detected in the cytoplasm was not a consequence of nuclear breakage during cell fractionation. Viral DNA synthesis was at least as efficient in enucleated cells as in intact cells; both strands of viral DNA were made in the enucleated cells and at least 60% of the viral genome was present in the newly synthesized DNA. Most of the DNA synthesis in the enucleated cells occurred within the first four hr, and there was no accumulation of viral DNA nine hr after infection. The results strongly suggest that viral DNA is synthesized in the cytoplasm and that induction of nuclear functions after infection is not required for the early events in the replication of ASV.

6189 ACCUMULATION OF WATER DURING TRANSFORMATION OF CELLS BY AN AVIAN SARCOMA VIRUS. *(E.)*

Bader, J. P. (Natl. Cancer Inst., Bethesda, Md.), D. A. Ray and N. R. Brown. *Cell* 3(3):307-313, 1974.

Potential size-related changes in the biophysical parameters of virus-transformed cells were investigated using cultured chick embryo (CE) cells transformed by the Bryan "high titer" strain of the Rous sarcoma virus (RSV-BH). The transformed cells were larger than nontransformed CE cells, as determined by direct measurements of diameter and volume. While the observed general increase in cell mass was insufficient to account fully for the increase in size, the RSV-BH-transformed cells were found to contain a greater proportion of water than the untransformed cells; this was reflected in a decrease in cellular density in the former. The observed changes in size, water content, and density were not found in cells infected with the Schmidt-Ruppin strain of avian sarcoma virus. CE cells infected with a mutant of RSV-BH were morphologically transformed at 37 C but not at 41 C; when shifted from 41 to 37 C, they became larger, accumulated more water, and banded higher in density gradients. These changes occurred without a requirement for new RNA or protein synthesis. The increase in the cellular water content may be an early event leading to changes in transcription and translation and culminating in malignancy in the RSV-BH-transformed cells.

6190 THE 60-70S RNA OF AVIAN SARCOMA AND LEUKOSIS VIRUSES--DISTRIBUTION OF CLASS *a* AND *b* SUBUNITS. *(E.)*

Duesberg, P. H. (Dep. Mol. Biol., Univ. California, Berkeley), P. K. Vogt and G. S. Martin. *Bibl Haematol* (39):462-473, 1973.

Two electrophoretically separable classes of subunits, *a* and *b*, were found after heat dissociation of the 60-70S RNA obtained from several avian sarcoma viruses [Schmidt-Ruppin Rous sarcoma virus (SR RSV-A), Prague strain RSV, RSV (RAV-0), virus B77]. Avian leukosis viruses (avian myeloblastosis virus, Carr-Zilber associated virus) yielded only class *b* subunits. The difference between sarcoma and leukosis viruses was not affected by the transformed or normal state of the host cell; e.g., class *a* subunits were present in progeny particles of a temperature-sensitive SR RSV-A mutant grown at the nonpermissive temperature for transformation. On the other hand, leukosis viruses released from sarcoma cells did not acquire class *a* subunits; e.g., fibroblast cultures transformed by the Bryan high-titer strain of RSV synthesized an excess of nonfocus-forming helper virus, but viral harvest contained few if any class *a* subunits. A similar preponderance of nonfocus-forming virus may also explain the lack of class *a* subunits in avian leukosis virus strain MC29, which is capable of forming neoplastic foci in fibroblast cultures. A connection between the ability to form foci in fibroblast cultures and the presence of class *a* subunits was further suggested by the observation that cloned stocks of SR RSV-A segregate nonfocus-forming virus which, together with its transforming potential, has lost class *a* subunits. These results are in agreement with the hypothesis that class *a* and *b* subunits of avian tumor virus RNA represent functionally specialized segments of the viral genome which may replicate independently.

6191 TYPE-C RNA VIRUS GENE EXPRESSION IN HUMAN TISSUE. *(E.)*

Strand, M. (Albert Einstein Coll. Med., New York, N.Y.) and J. T. August. *J Virol* 14(6):1584-1596, 1974.

Partially purified fractions of human tissues were analyzed by competition radioimmunoassay for the presence of two of the principal structural components of type-C RNA viruses: the major core protein (p27-p30) and the major envelope glycopeptides (gp69/71). Screening of tissues was carried out by use of a heterologous assay system of ^{125}I-labeled Rauscher murine virus p30 antigen and anti-RD 114 virus serum, which was found to detect a class of interspecies determinants common to murine, feline, and primate viruses. A competitor with the same apparent affinity for antibody binding as that of purified viral core proteins was found in relatively high concentrations in tissues from patients with systemic lupus erythematosus, in some neoplastic tissues, and in some normal human tissues. This competitor from a lupus spleen was chromatographed on phosphocellulose and showed size fractionation during gel filtration similar to known p27-p30 viral proteins. An immunologically reactive protein was also demonstrated by immunodiffusion and by immunoprecipitation of ^{125}I-labeled human protein with anti-RD 114 p28 serum. Analysis of these human competitor proteins with homologous assay systems of viral core proteins and corresponding antisera showed that all, including the normal tissue extracts, appear similar to core proteins of known viruses, especially the RD 114 and woolly monkey species. The data suggest that many, if not all, humans harbor at least part of the genome of one or more type-C viruses, the properties of which are similar to those of viruses of other mammalian, particularly primate, species.

6192 TYPE C VIRAL EXPRESSION IN PRIMATE TISSUES. *(E.)*

Sherr, C. J. (Natl. Cancer Inst., Bethesda, Md.), R. E. Benveniste and C. J. Todaro. *Proc Natl Acad Sci USA* 71(9):3721-3725, 1974.

Nucleic acid sequences homologous to a [^{3}H]DNA transcript prepared from an endogenous baboon type C virus were detected in both the cellular DNA and RNA of several different Old World monkeys. Tissues from two baboon species, patas, rhesus, stumptail, and African green monkeys and a human spleen were investigated. Competitive radioimmunoassays for the major viral protein (p30) of baboon type C viruses detected viral antigen in a normal stumptail spleen and a rhesus ovarian carcinoma. The p30 antigen from these tissues was closely related by several immunologic criteria to the p30 protein of baboon type C viruses. The findings suggest that the primary amino-acid sequences of the baboon, rhesus and stumptail p30 proteins have not undergone extensive evolutionary modification; the results also indicate that normal primate tissues transcribe endogenous viral-specific RNA and translate at least one viral structural protein.

6193 C-TYPE VIRUSES IN SYSTEMIC LUPUS ERYTHEMATOSUS. *(E.)* Lewis, R. M. (Tufts Univ. Sch. Med., Boston, Mass.), W. Tannenberg, C. Smith and R. S. Schwartz. *Nature* 252(5478):78-79, 1974.

Preliminary evidence linking the SP 104 tumor virus to systemic lupus erythematosus in humans is presented. An antibody to the SP104 virus was induced in New Zealand rabbits using purified C particles isolated from the fluid of cultured SP 104 cells. Purified C-type virus was disrupted with ether and injected s.c. Ten days after two injections of the virions, serum was collected and absorbed sequentially with fetal calf serum, normal murine spleen cells and blood lymphocytes from normal human subjects. A globulin-rich fraction of the absorbed serum was conjugated to fluorescein isothiocyanate. Lymphocytes of normal subjects failed to react with the conjugate but typical and brilliant membrane fluorescence was seen on the lymphocytes of 5/6 patients with system lupus erythematosus (SLE). Serum from a 12-yr-old girl with untreated SLE was treated with ammonium sulphate, conjugated to fluorescein isothiocyanate, and used to stain the blood lymphocytes of several subjects in the same manner as the rabbit serum. The serum failed to bind to the lymphocytes of six normal subjects, or of six patients with diseases other than SLE. Typical membrane staining was found, however, with eight of ten patients with SLE. The immunoglobulin fraction of the serum of the 12-yr-old girl was absorbed with pelleted C-type particles obtained from the SP 104 culture. The absorbed material, after conjugation to fluorescein isothiocyanate, failed to stain the lymphocytes of five/eight patients with SLE. The results indicate that more than one SLE-related antigen may appear on the membranes of circulating lymphocytes during the acute phase of the illness. It is suggested that the lymphocytes of patients with SLE express an antigenic determinant shared by C-type RNA viruses produced by the SP 104 tumor. Results with the rabbit antibody, which is directed against purified virus, suggest the presence of a virion determinant on the cytoplasmic membranes of lymphocytes of patients with SLE.

6194 CAFFEINE INHIBITS INDUCTION OF ENDOGENOUS C-TYPE VIRUS. *(E.)* Yoshikura, H. (Inst. Radium Biol., Orsay, France). *Nature* 252(5478):71, 1974.

The MLg cell line originating from a newborn ddY mouse was used to study the effects of caffeine on the induction of endogenous C-type virus. MLg cells inoculated in 60 mm plastic Petri dishes were treated with IUdR (5-50 µg/ml) for 24 hr; the medium was removed and untreated MLg cells were laid over the IUdR-treated cells. For IUdR concentrations of 5, 20 and 50 µg/ml, the mean plaque number/dish was 19, 73, and 125, resp. Other MLg cells were treated with caffeine (0.625 to 5 mM) for 24 hr before or after IUdR treatment (25 µg/ml for 24 hr). Twenty-four hr after the removal of IUdR, the cultures were overlaid with normal MLg cells. Caffeine treatment after IUdR treatment significantly inhibited virus induction; a proportional relation was observed between caffeine concentration and the inhibition of induction of the C-type virus. When MLg cells were treated with caffeine (1.25 to 5 mM) for 24 hr before or after infection with Friend murine leukemia virus, the treatment had no effect on exogenous virus infection. Since caffeine affects neither exogenous virus infection nor incorporation of ^{125}IUdR (data not shown), it is suggested that caffeine may interfere with events specific to virus induction. It is further suggested that IUdR may induce local distortion of DNA and caffeine may then interact with such distorted regions of the DNA so as to prevent viral gene activation.

6195 INTERACTION BETWEEN MURINE TYPE-C VIRUS RNA-DIRECTED DNA POLYMERASES AND RIFAMYCIN DERIVATIVES. *(E.)* Wu, A. M. (Bionetics Res. Lab., Bethesda, Md.) and R. C. Gallo. *Biochim Biophys Acta* 340(4):419-436, 1974.

Previous studies indicated that some rifamycin SV derivatives are inhibitors of purified murine type C virus RNA-directed DNA polymerase, although other polymerases are also affected. These compounds were grouped into three classes (A, B, and C) based on their relative inhibitory effect on the viral polymerase. The effective dose for 50% inhibition by the three classes is 5, 20, and 100 µg/ml, resp. Further experiments were conducted to determine: (1) if the effective inhibitors bind to the viral polymerase, (2) if binding occurs, how it compares with binding to other polymerases, and (3) whether binding is required for inhibition of enzyme activity. Results show that the inhibitory effect is independent of the choice of template-primer but is sensitive to the presence of nonionic detergent. Potent inhibitors bind to the viral enzyme as shown by cosedimentation of the inhibitor with purified enzyme during glycerol gradient centrifugation. At least two molecules of potent (class C) inhibitors bind to each molecule of the viral enzyme. This binding is reversible with a relatively low concentration of Triton X-100 (0.05%) as judged by a complete restoration of the enzyme activity. Similar results were found with bacterial DNA polymerases; however, binding was much firmer between *Escherichia coli* RNA polymerase and rifamycin derivatives since only 20% recovery of enzyme activity was obtained at a Triton X-100 concentration as high as 5%. With respect to the purified viral DNA polymerase, the rifamycin derivatives interact in decreasing order with the free form of the enzyme, the enzyme-template-primer complex, the enzyme-substrate complex and the initiation complex. The inhibitors were noncompetitive with the deoxyribonucleoside triphosphate substrates with K_i equivalent to 10^{-6} M. The inhibition appears to be at the step before initiation. Therefore, conditions that facilitate initiation interfere with the effectiveness of these compounds, and the order of addition of each component becomes critical in assessing their relative inhibitory activity.

6196 INDUCTION OF TYPE-C RNA VIRUS BY CYCLOHEXIMIDE: INCREASED EXPRESSION OF VIRUS-SPECIFIC RNA.

(E.) Aaronson, S. A. (Natl. Cancer Inst., Bethesda, Md.), G. R. Anderson, C. Y. Dunn and K. C. Robbins. *Proc Natl Acad Sci USA* 71(10): 3941-3945, 1974.

Several chemicals which inhibit different steps in the synthesis of proteins in eukaryotes induce type-C virus in virus-negative mouse cells; the mechanism of action of one of these chemicals, cycloheximide, was studied. Virus activation in BALB/c mouse embryo cells in response to cycloheximide (10 µg/ml) was transient, with virus release occurring primarily within the first 12-24 hr following drug exposure. Analysis of the cellular virus-specific RNA revealed an absolute increase in the viral RNA concentration in the cycloheximide-treated cells as compared with untreated cells. This was blocked by simultaneous exposure of the cells to actinomycin D. Inhibition of RNA synthesis during, but not subsequent to, cycloheximide exposure also prevented virus activation. These findings indicate that virus induction by cycloheximide required *de novo* RNA synthesis during, but not after, drug exposure, and suggest that the RNA species required may be that of the virus itself. The data are consistent with the hypothesis that translational inhibitors prevent the synthesis of a labile protein whose normal action is to inhibit viral RNA transcription or to cause degradation of the viral RNA.

6197 IMMUNOLOGICAL CROSS-REACTIONS BETWEEN TWO LOW-MOLECULAR-WEIGHT POLYPEPTIDES FROM A MURINE TYPE C VIRUS.

(E.) Parks, W. P. (Natl. Cancer Inst., Bethesda, Md.), E. M. Scolnick, M. C. Noon and C. J. Watson. *J Virol* 14(3):430-433, 1974.

Competition radioimmunoassays showed that two low molecular-weight type-specific virion polypeptides, p10 and p12, of Kirsten murine leukemia virus (MuLV) are immunologically related. Competition assays were carried out at a serum concentration which precipitated 35-50% of the labeled protein. With ^{125}I-labeled Kirsten p12, competition between this polypeptide and Kirsten p10 yielded essentially identical displacement curves in terms of sensitivity of detection (1-3 mg/assay), slope of curve, and final degree of displacement. Labeled Kirsten p10 displaced labeled antigen more efficiently than did p12 Kirsten polypeptide (detected at < 1 ng assay and displacement up to 92%), but both were highly related as judged by this method. Both the p10 and p12 reactivities were detected as discrete peaks in competition assays with individual fractions from guanidine hydrochloride chromatography and polyacrylamide gel-eluted fractions. Similar results were obtained with purified p10 and p12 from three different Kirsten MuLV preparations grown in 3T3 cells. A xenotropic MuLV strain from BALB/c 3T3 did not react in either assay; furthermore, neither p30 (group-specific) nor gp70 from Kirsten MuLV competed in the p12 and p10 competition assays. This study, together with previous work, suggests that a product-precursor relationship may account for the observed immunological cross reaction between low molecular weight polypeptides of type C viruses.

6198 ANALYSIS OF CHROMOSOMES OF SYRIAN HAMSTER CELLS TRANSFORMED WITH HUMAN CYTOMEGALOVIRUS.

(E.) Nachtigal, M. (Milton S. Hershey Med. Cent., Pennsylvania State Univ., Hershey), T. Albrecht and F. Rapp. *Intervirology* 4(2):77-90, 1974.

A Syrian hamster embryo cell line derived after transformation by human cytomegalovirus (CMV) and six cell lines derived from three tumors and a metastasis induced by transplantation of the *in vitro* transformed cells into animals were analyzed with cytogenetic techniques. A spontaneously-transformed hamster cell line was included in the study. The CMV-transformed cell lines grown *in vitro* kept diploid or near-diploid chromosome numbers and contained 2-20% cells with unstable structural chromosome aberrations. These were predominantly dicentrics and sometimes exhibited abnormal heterochromatic regions. In advanced passages one marker chromosome (mar 1) was found by Giemsa banding. The cell lines derived from tumors were near-diploid, mixoploid, or heteroploid with chromosome numbers in the triploid-tetraploid range. They contained 24-40% cells with unstable chromosome aberrations including dicentrics, double-minutes, and acentrics. A marker chromosome similar to mar 1 was found in three of these cell lines and another marker was present in four cell lines. A reduction in the number of acrocentric (D) chromosomes was noticed in all cell lines. Spontaneously-transformed cells were hyperdiploid and contained acentrics and double-minutes but no dicentrics. These results suggest that CMV-transformed cells maintained an abnormal but stable chromosome complement *in vitro*, and that these became unstable when derived from tumors. Some marker chromosomes seemed stable.

6199 EPSTEIN-BARR VIRUS INFECTION IN THE NEONATAL PERIOD AND IN CHILDHOOD.

(E.) Joncas, J. (Inst. Microbiol. Hyg. Montreal, Canada), J. Boucher, M. Granger-Julien and C. Filion. *Can Med Assoc J* 110(1):33-37, 1974.

In an attempt to detect and characterize congenital, neonatal, and early childhood Epstein-Barr virus (EBV) infections, a prospective seroepidemiologic study was undertaken on 112 neonates and their mothers, 25 additional newborns undergoing exchange transfusion, 114 randomly-selected hospitalized patients aged 0-3 yr and 102 siblings and parents of these infants. Leukocyte culture was attempted in all the newborns and in 25 pre- and post-transfusion patients. Seroconversion in the absence of apparent illness occurred in 6/60 EBV-negative children; follow-up of these converters and their families revealed no case of infectious mononucleosis. Among 42 EBV-positive patients was a three-yr-old girl who developed characteristic heterophil agglutinins; her EBV antibody titer rose from 1/20 on admission to 1/320. Seroconversion from positive to negative was observed in three children. In 3/112 cases, newborn EBV antibody titers were significantly higher than the maternal antibody titers. Two permanent lymphoblastoid cell lines were established from newborns following exchange transfusion. Although both infants became EBV-negative by immunofluorescence at age three and six months, EBV antibodies were detected by complement fixation in both cases at age six and nine months. These findings suggest the possibility of abortive primary EBV infection in early life. Persistence of EBV antibodies in two newborns may represent instances of congenital infections.

6200 *IN VITRO* TRANSFER OF CELLULAR IMMUNITY AGAINST NASOPHARYNGEAL CARCINOMA USING TRANSFER FACTOR FROM DONORS WITH EPSTEIN-BARR VIRUS ANTIBODY ACTIVITY. *(E.)* Brandes, L. J. (Dep. Med., Univ. Manitoba, Winnipeg, Canada) and G. J. Goldenberg. *Cancer Res* 34(11):3095-3101, 1974.

The peripheral leukocyte migration inhibition assay was adapted as an *in vitro* assay of cell-mediated immunity to a soluble antigen extract of nasopharyngeal carcinoma and as an assay for transfer activity in this system. Two groups, each consisting of ten normal subjects who were either Epstein-Barr virus positive (antibody titers of 1:16 or greater) or Epstein-Barr virus negative (antibody titers of less than 1:4), were tested with the leukocyte migration inhibition assay against nasopharyngeal carcinoma antigen at concentrations of 50, 100, and 175 µg/ml. Inhibition of migration greater than 20% (in the presence of antigen) compared to control values (in the absence of antigen) was considered a positive result. The ten normal individuals with positive Epstein-Barr virus antibody titers demonstrated leukocyte migration inhibition of 36.8% at a nasopharyngeal carcinoma antigen concentration of 50 µg/ml, compared to a migration inhibition of 13.8% in ten Epstein-Barr virus negative normal subjects. Four nasopharyngeal carcinoma patients in remission demonstrated a migration inhibition of 28.8% when their leukocytes were incubated in the presence of nasopharngeal carcinoma antigen while four patients in relapse did not demonstrate migration inhibition. Transfer factor derived from Epstein-Barr virus positive donor leukocytes converted leukocytes of Epstein-Barr virus negative normal recipients to reactivity against the nasopharyngeal carcinoma antigen in seven of nine cases. The ability of transfer factor to convey reactivity was specific and correlated with Epstein-Barr virus humoral immunity in the transfer factor donor. Transfer factor conferred similar reactivity upon the leukocytes of two of four nasopharyngeal patients in relapse. The presence of cross-reactivity against nasopharyngeal carcinoma antigen not only in Epstein-Barr virus positive normal subjects but also in nasopharyngeal carcinoma patients suggests an immune response against an oncogenic virus. This finding may provide an additional, although indirect, link between Epstein-Barr virus and nasopharyngeal carcinoma.

6201 ERYTHROPOIETIN RESPONSIVENESS OF DIFFERENTIATING FRIEND LEUKAEMIA CELLS. *(E.)* Preisler, H. D. (Mt. Sinai Sch. Med., New York, N.Y.) and M. Giladi. *Nature* 251(5476):645-646, 1974.

The relationship between differentiation and erythropoetin (Ep) responsiveness was studied in Friend leukemia cells (FLC, clone 745A). Dimethylsulfoxide (DMSO)-treated cells were grown in suspension culture to which Ep (human urinary Ep, 75.4 IV/mg) was added after various intervals. The rate of heme synthesis was assayed after the addition of Ep. Ep significantly stimulated the rate of heme synthesis in DMSO-treated cells, but not in cells which were not pretreated with DMSO; it did not affect the rate of increase in cell numbers. Ep also increased the rate of leucine and uridine incorporation into the cells, as well as the rate of DNA synthesis. The addition of Ep to cells which had not been treated with DMSO decreased the rate of thymidine incorporation into the cellular DNA. The increase in heme synthesis was observed when EP was added to cultures which had been growing in the presence of DMSO for three or more, but not fewer, days. Heme synthesis was reduced when Ep was added at the time of seeding in the presence of DMSO. Human Ep did not differ in effect from sheep Ep. The results suggest that erythroleukemic cells, and perhaps leukemic cells in general, possess the genetic information needed for the development of responsiveness to normal physiological regulators and that under appropriate conditions this information can be expressed.

6202 CHARACTERIZATION OF REVERSE TRANSCRIPTASE AND RNASE H FROM FRIEND-MURINE LEUKEMIA VIRUS. *(E.)* Moelling, K. (Robert Koch Inst., Berlin, West Germany). *Virology* 62(1):46-59, 1974.

In order to compare directly the properties of reverse transcriptase obtained from Friend murine leukemia virus (Fr-MuLV) of mammalian origin to that of avian myeloblastosis virus (AMV), identical purification procedures were used. Fr-MuLV reverse transcriptase was isolated from Nonidet P-40-treated virus by means of diethylaminoethyl cellulose, followed by CM-Sephadex or phosphocellulose chromatography, and additional glycerol density-gradient centrifugation. The sedimentation coefficient (4.8 S), as determined in the presence of high salt, was consistent with a single 84,000 polypeptide as deduced by polyacrylamide gel electrophosesis in the presence of SDS. DNA polymerase and RNase H were assayed and extracted. Reverse transcriptase activity was traced throughout the parallel purification with poly(A)·oligo(dT)$_{10}$ as template and RNase H with the natural DNA-RNA hybrid. Endogenous DNA polymerase activity and RNase H activity were found to be associated with purified Fr-MuLV after disruption and both activities were chromatographed throughout all purification procedures without preferential loss of RNase H. The origin of the RNase H was concluded to be viral, due to its inability to attack ribonucleotides in closed circular DNA-RNA-hybrid structures as cellular RNase H did. The RNA-dependent DNA transcription of AMV RNA with the purified Fr-MuLV enzyme was half as efficient as with AMV DNA polymerase similarly prepared. The only significant enzymological differences between the reverse transcriptase RNase H complexes was that the Fr-MuLV RNase H showed a 4- to 5-fold increased efficiency with Mn^{2+} as compared to Mg^{2+}. The Fr-MuLV polypeptide was also determined to be larger than the AMV polypeptide. The author concluded that the enzymes from avian and mammalian origin perform the same biochemical functions, respond well to natural RNA and contain RNase H even though they are structurally and antigenically different.

6203 PURIFICATION OF *HERPESVIRUS SAIMIRI* DNA. *(E.)* Laufs, R. (Inst. Hyg., Univ. Gottingen, West Germany) and B. Fleckenstein. *Bibl Haematol* (39):457-461, 1973.

In order to label *Herpesvirus saimiri* DNA, 10 μCi/ml ^{3}H-thymidine was added to owl monkey kidney cultures 24 hr after infection. Sedimentation of labeled virus in sucrose gradient yielded a peak of radioactivity that correlated with the peak of infectivity. The material of the virus peak was DNase resistant, whereas the trichloracetic acid-precipitable radioactive material was not. In potassium tartrate, the peak infectious fraction banded at a density of 1.18 g/ml; inoculation of this fraction into a cotton-topped marmoset induced a typical malignant lymphoma. After the peak sucrose gradient fractions were treated with Nonidet P40, electron microscopy showed the presence of nucleocapsids with the typical structure of herpesviruses. The viral DNA in the nucleocapsid fractions had the same molecular weight as bacteriophage T 4 DNA (1.1 x 10^8 daltons). In CsCl, the nucleocapsid DNA banded in two peaks, one unidentified and the other corresponding to *Micrococcus lysodeikticus* DNA, which was added as a marker of viral DNA. Buoyant density determinations of *Herpesvirus saimiri* DNA in CsCl were repeated with herpes simplex DNA, *Micrococcus lysodeikticus* DNA, and vero-cell DNA added as markers. Results showed that the exact density of purified *Herpesvirus saimiri* DNA, is 1.729 ± 0.002 g/ml. This corresponds to a base composition of 70% guanine and cytosine.

6204 REQUIREMENT OF ARGININE FOR THE REPLICATION OF MAREK'S DISEASE HERPES VIRUS. *(E.)* Mikami, T. (Sapporo Med. Coll., Japan), M. Onuma and T. T. A. Hayashi. *J Gen Virol* 22(1):115-128, 1974.

The arginine requirements for the replication of Marek's disease herpes virus (MDHV) was studied in cultured Japanese quail embryo cells (QEFC). Arginine was found to be essential for the replication of MDHV in the QEFC cultures. The plaque-forming activity of MDHV was inhibited by the omission of arginine from the culture medium and resumed following the addition of the amino acid to the arginine-deprived cultures. The MDHV genome survived in a recoverable form in infected cultures kept in arginine-deprived medium for up to 10 days. Determination of the specific arginine-dependent period during virus replication demonstrated that the presence of the amino acid was required during the period beginning about 12 hr after virus inoculation. Omission of arginine from the culture medium did not affect virus adsorption, penetration, or the synthesis of viral DNA, but it did prevent the formation of the viral structural protein which is detectable using immunofluorescent antibody (FA) techniques. The results indicate that arginine is required for the synthesis of the late protein which is detected by FA staining which is necessary for MDHV replication.

6205 TRANSIENT INFECTIVITY AND HETEROKARYON FORMATION IN HAMSTER CELL CULTURES INOCULATED WITH CELL-ASSOCIATED STOCKS OF MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEYS. *(E.)* Witter, R. L. (Reg. Poult. Res. Lab., East Lansing, Mich.) and J. M. Sharma. *J Natl Cancer Inst* 53(6):1731-1742, 1974.

The nature of the interaction between Marek's disease virus (MDV) and herpesvirus of turkeys (HVT) and mammalian cells was studied. Virus infectivity persisted for up to ten serial passages in primary hamster kidney (HK) cultures inoculated with 4.3 x 10^4 plaque-forming U of cell-associated MDV or 8 x 10^4 plaque-forming U of cell-associated HVT. Maintenance of viral infectivity showed no correlation with the number of plaque-forming U inoculated but was correlated with the persistence of at least 10^5 inoculum cells/culture. Ablation of infectivity by treatment with cytotoxic anti-avian cell antibody and failure to infect HK cultures with high doses of cell-free MDV or HVT also indicated that infection was solely associated with inoculum-derived avian cells. Heterokaryons containing avian and hamster nuclei as well as viral antigens were observed in HK cultures inoculated with virus-infected avian cells. Because infection of HK cells was transient, the practical significance of heterokaryon formation on virus replication in the cells is apparently negligible. It is concluded that HK cells do not support persistent infection with MDV and are probably refractory to infection with these viruses.

6206 ULTRASTRUCTURAL STUDY OF HERPES SIMPLEX VIRUS INFECTION OF MOUSE CELLS CHRONICALLY PRODUCING RAUSCHER LEUKEMIA VIRUS. *(E.)* Andrese, A. P. (Natl. Cancer Inst., Bethesda, Md.), G. F. Vande Woude and M. J. Walling. *J Natl Cancer Inst* 53(4):1169-1173, 1974.

Mouse cells (JLSV9) chronically infected with Rauscher Leukemia virus (JLSV9-RLV) were superinfected with low multiplicities of herpes simplex virus subtype-1 (HSV-1) and examined 12 and 24 hr later by electron microscope to determine whether both HSV-1 and RNA tumor virus could be detected in a single cell. In several cells, both viruses were observed in various stages of development. Early stages of herpesvirus development were indicated by condensation and margination of chromatin as well as by intranuclear nucleocapsids, while a late stage of C-type particle budding was observed and what may have been the initiation of C-type budding was indicated. Large numbers of double-enveloped herpes-like particles were observed in the 24-hr infected JLSV9-RLV cultures, but never in the JLSV9 cells. The results indicate that there is no apparent restriction of HSV-1 replication in JLSV9-RLV cells.

6207 CONDITIONS FOR OPTIMAL MTV SYNTHESIS *IN VITRO* AND THE EFFECT OF STEROID HORMONES ON VIRUS PRODUCTION.

(E.) Dickson, C. (Dep. Zool., Univ. California, Berkeley), S. Haslam and S. Nandi. *Virology* 62(1):242-252, 1974.

The DNA polymerase assay was used with synthetic template/primers to measure the synthesis of mouse mammary tumor virus (MTV) obtained from the tissue culture fluid of primary mouse mammary tumor cells *in vitro*. A high level of DNA polymerase activity was obtained with the synthetic template/primer $rA:dT_{10}$ and a low level was obtained with $dA:dT_{10}$. The DNA polymerase activity was associated with particles exhibiting the physical, morphological, and immunological properties of MTV; there was a linear relationship to protein concentration up to 40 μg/ml. A high cell seeding density was required for optimal MTV synthesis, and synthesis was stimulated by a variety of steroid hormones; dexamethasone was the most potent, followed by hydrocortisone, corticosterone, aldosterone, and deoxycorticosterone. In the presence of fetal calf serum, insulin had a slight stimulatory effect. An increase in the rate of MTV synthesis was detectable 8-10 hr after exposure of the cells to hydrocortisone or other steroid hormone systems; these systems had no detectable effects on the overall rate of DNA, RNA, or protein synthesis. The results suggest that the rate of MTV synthesis is controlled *via* a receptor mechanism acting either indirectly on a critical cell function required for MTV synthesis or directly on an integrated viral genome.

6208 A CELL LINE DERIVED FROM BALB/3T3 CELLS: A SUBSTRATE FOR MURINE LEUKEMIA VIRUS ASSAY AND FOR QUANTITATIVE STUDIES ON THE TRANSFORMATION OF CELLS.

(E.) Hackett, A. J. (Nav. Biomed. Res. Lab., Univ. California, Berkeley), S. S. Sylvester and M. Calvin. *Collect Pap Annu Fundam Cancer Res* 25:300-302, 1974.

The cell line, UC1-B, which is derived from Balb/3T3 and undergoes transformation upon inoculation with murine leukemia virus (MuLV) was studied. Results indicated that UC1-B cells were as sensitive to MuLV infection as A31 cells. Electron microscopy of Moloney leukemia virus (MLV) infected cells showed two forms of viral maturation that occurred simultaneously: the typical C-type virus buds from the plasma membrane and a bizarre type of maturation. Aberrant budding at the periphery of a cytoplasmic vacuole led to the development of unusual, cylindrical particles or structures. Both typical and atypical virus formations were present in 95% of the cells observed. The effect of di-methyl-benzyl rifampicin or transformation of UC1-B cells by MLV infection was also studied. Although transformation was inhibited (6 μg/ml) to 75-80% of the nondrug-treated cells, leukemia virus replication was not inhibited. These results suggested that the intracellular action of the drug was more complex than a simple block of viral RNA-directed DNA polymerase. UC1-B provides a means for direct quantitative study of transformation.

6209 MURINE SARCOMA VIRUS-INDUCED CYTOPATHOLOGICAL EFFECTS IN MOUSE CELLS MADE RESISTANT TO 6-AZAURIDINE.

(E.) Long, C. W. (Flow Lab., Inc., Rockville, Md.), E. Good, R. V. Gilden and K. Rand. *J Gen Virol* 23(2):185-189, 1974.

A variety of drug resistant mouse cells with specific enzyme deficiencies were tested for their ability to support the multiplication of leukemia and sarcoma viruses. Two sarcoma viruses, the Rauscher pseudotype of the Moloney strain [M-MSV(RLV)] and the Moloney strain of murine sarcoma virus [M-MSV(MLV)] and three leukemia viruses [Rauscher (RLV), Friend (FLV), and Moloney (MLV)] were used to infect secondary Swiss Mouse embryo cells (MEC), random bred Swiss mouse embryo 3T6 cells, and three clonal derivatives of 3T6 cells made resistant to 6-azauridine (6-AU). The 6-AU-resistant 3T6 cells were found to undergo syncytium formation upon infection with M-MSV(RLV) and M-MSV(MLV). Cultures with numerous syncytia were negative in hemadsorption tests with guinea pig red blood cells, electron microscopic examination of the cultures revealed only C-type particles, and syncytia formation by M-MSV(RLV) on 6-AU-resistant cells was inhibited by antiserum to RLV in exact parallel to the inhibition of reverse transcriptase and the induction of gs antigen. In addition, culture fluids from the 6-AU-resistant cells induced no syncytia on MEC and 3T6 cells, although these fluids did produce syncytia on 6-AU-resistant cells. No syncytia were produced on 6-AU-resistant cells infected with leukemia virus alone or β-propiolactone-inactivated M-MSV(RLV). The results suggest that the formation of syncytia was related to sarcoma virus replication.

6210 CHARACTERIZATION OF THE MAJOR VIRAL POLYPEPTIDE IN CELLS TRANSFORMED BY WILD-TYPE AND TEMPERATURE-SENSITIVE MURINE SARCOMA VIRUS.

(E.) Spira, G. (Baylor Coll. Med., Houston, Tex.), G. R. Dreesman, M. Benyeshi-Melnick, S. Kit and K. D. Somers. *Intervirology* 4(2):99-109, 1974.

Extracts of Moloney murine sarcoma virus (M-MSV)-transformed rat cells were analyzed by a radioimmunoassay procedure that detects the 30,000-dalton major virus structural polypeptide (p30). With the appropriate choice of antiserum and competing protein, type-specific p30 antigenic determinants of Moloney leukemia virus were detected in extracts of producer and nonproduer M-MSV-transformed cells. The specific antigenic determinants were expressed at permissive and restrictive temperatures in cells transformed by a cold-sensitive M-MSV mutant, suggesting that expression of type-specific p30 antigens is not required for maintenance of the transformed phenotype. In parallel experiments, Rauscher leukemia type-specific p30 antigenic determinants were detected in extracts of nonproducer M-MSV-transformed cells superinfected with Rauscher leukemia virus. The intracellular p30 antigen in extracts of wild-type and mutant M-MSV-transformed cells was characterized by Sephadex chromatography and isoelectric focusing. Extracts of M-MSV-transformed cells contained a single peak of p30 antigenic activity with a molecular weight of approximately 30,000 daltons and a isoelectric point of 6.0, suggesting that the polypeptide in cellular extracts was similar in molecular weight and isoelectric point to the corresponding protein isolated from purified Moloney murine sarcoma-leukemia virus.

6211 EFFECTS OF ACTINOMYCIN D ON EARLY STEPS OF REPLICATION *IN VITRO* OF MURINE SARCOMA VIRUS (MOLONEY).

(E.) Godard, C. (Hosp. St.-Louis, Paris, France), J. Robin and M. Boiron. *J Gen Virol* 22(2):293-296, 1974.

The sensitivity of the early steps in the *in vitro* replication of murine sarcoma virus (Moloney) (M-MSV) to actinomycin D was investigated. M-MSV infected BALB/c mouse embryo cells were treated for six hr with actinomycin D (0.25 μg/ml) in growth medium. The production of infective virus was inhibited by actinomycin D provided the cells were exposed to the latter not later than 2.5 hr after virus infection. The release of virus particles into the supernatant fluids of infected cultures and the production of infective centers were also reduced or prevented by treatment with actinomycin D, as was cell conversion. The early actinomycin D-sensitive step in the virus replication cycle may be the synthesis of DNA-provirus. Actinomycin D may also act by preventing the transcription of DNA-provirus.

6212 EFFECTS OF STAS JLSV$_5$ AND SILICOTUNGSTATE ON THE REPLICATION OF MURINE SARCOMA VIRUS (MOLONEY) AND POLIOVIRUS.

(E.) Jasmin, C. (Hosp. Paul Brousse, Villejuif, France), J. C. Chermann, N. Raybaud, D. Bucchini, O. Jarrett, M. Raynaud, G. Mathé and F. Plata. *Bibl Haematol* (39):381-388, 1973.

STAS JLSV$_5$, a preparation obtained from the supernatant of murine cells chronically infected with Rauscher leukemia virus, and silicotungstate (ST) were studied for their antiviral effects. Both products inhibited replication of Moloney murine sarcoma virus in mouse embryonic fibroblasts, but noticeable differences were observed in the timing of action. STAS JLSV$_5$ (0.2 mg/ml for three days) was inhibitory when added to the medium from 0 to more than 24 hr after virus infection. ST (0.2 mg/ml for three days) was active when added prior to virus inoculation and until +15 hr postinfection. Additional experiments demonstrated that ST inhibited the stimulation of DNA synthesis induced by murine sarcoma virus and blocked cellular infection. Only STAS JLSV$_5$ inhibited replication of poliovirus type I in actinomycin D-treated KB cells; ST was completly inactive in this system. These results indicate that STAS JLSV$_5$ contains an antiviral component capable of inhibiting both oncogenic and nononcogenic viruses. Studies to characterize the component and to determine its spectrum of activity *in vitro* and *in vivo* are in progress.

6213 ALTERATIONS IN THE PATTERN AND SYNTHESIS OF GANGLIOSIDES IN TUMORIGENIC VIRUS-TRANSFORMED CELLS.

(E.) Brady, R. O. (Natl. Inst. Neurol. Dis. Stroke, Bethesda, Md.) and P. H. Fishman. *Cold Spring Harbor Conf Cell Proliferation* 1:505-515, 1974.

The composition and metabolism of the gangliosides in tumorigenic DNA and RNA virus-transformed cells was studied. Normal and virus-transformed lines were derived from AL/N, Balb/c, and Swiss mice. Lipids were isolated and analyzed using standard extraction and thin-layer chromatographic procedures, and the determinations were verified by gas-liquid chromatography of the glycolipids. The ganglioside patterns in the tumorigenic DNA and RNA virus-transformed cell lines were markedly simplified compared to those of their respective contact-inhibited parental lines. This change in the transformed cells was primarily due to a decrease in the activity of hematoside:N-acetylgalactosaminyltransferase, which catalyzes an early step in the synthesis of the oligosaccharide chain of gangliosides. Recently, a block in galactosyltransferase activity was observed in Balb/c cells transformed with the Kirsten strain of murine sarcoma virus; there was no decrease in the activity of hematoside:N-acetylgalactosaminyltransferase in these cells. In contrast to the virus-transformed cells, spontaneously transformed tumorigenic cells showed ganglioside patterns similar to those seen in the parental lines. Also in contrast to the virus-transformed cells, some disialoganglioside appeared to be synthesized in cells which had been chemically transformed *in vitro*; however, the virus- and chemically-transformed cells showed similar increases in ganglioside G_{M2}. These cell systems are of value in the investigation of the metabolic and compositional alterations which are the consequences of viral carcinogenesis.

6214 THE 7S RNA COMMON TO ONCORNAVIRUSES AND NORMAL CELLS IS ASSOCIATED WITH POLYRIBOSOMES.

(E.) Walker, T. A. (Natl. Jew. Hosp. Res. Ctr., Denver, Colo.), N. R. Pace, R. L. Erikson, E. Erikson and F. Behr. *Proc Natl Acad Sci USA* 71(9):3390-3394, 1974.

7S RNA isolated from mouse embryo fibroblasts and HeLa S_3 cells and purified by methylated-albumin-Kieselguhr column chromatography was studied to determine its location in the cell. Fractions of whole cell, polyribosome supernatant and polyribosome pellet were examined for 7S RNA content and the polyribosome pellet was found to contain about half of the cellular 7S RNA. HeLa cells were treated with 12% Me_2SO or Me_2SO plus cycloheximide (100 μg/ml as the control). The following amounts (expressed as a percentage of the total 7S or 5S RNA in the three fractions) were recorded in the control group for 7S and 5S RNA, resp.: total ribosomes (49% and 47%), 100-200S region of gradient (20% and 15%), and 80S monomers (31% and 38%). The following percentages were recorded in the Me_2SO-treated cells for 7S and 5S, resp: total ribosomes (15% and 9%), 100-200S region (27% and 19%), and 80S monosomes (58% and 72%). The loss of 7S RNA from the polyribosome region of the sucrose gradient paralleled dissociation of the polyribosomes by Me_2SO. Distribution of the 5S rRNA revealed disaggregation of 80% of the polyribosomes and a 70% loss of 7S RNA. Previous findings indicated quantitative correspondence of the 7S RNA with mRNA. These indications and the results presented suggest that 7S RNA interacts with mRNA in some respect and could account for its inclusion within oncornavirions where the genome (7S RNA) may serve as mRNA shortly before virus assembly.

6215 REPLICATION OF ONCORNAVIRUS-LIKE PARTICLE IN HUMAN BREAST CARCINOMA CELL LINE, MCF-7.

(E.) McGrath, C. M. (Div. Biol. Sci., Michigan Cancer Found., Detroit), P. M. Grant, H. D. Soule, T. Glancy and M. A. Rich. *Nature* 252(5480):247-250, 1974.

The density distribution of RNA-containing particles released from MCF-7 human breast carcinoma cells during a 48-hr labeling period at 25 C showed a major particle population at 1.17 g/ml and a minor population at 1.23-1.25 g/ml. When the cells were labeled at 37 C, only the particles of density 1.23-1.25 g/ml were recovered. Four RNA populations were identified by gel electrophoresis in a particle population of density of 1.16-1.18 g/ml; the molecular weights of these RNAs ranged from 1×10^6-12×10^6 daltons. The major RNA species from the MCF-7 cells consistently displayed reverse transcriptase activity using endogenous RNA as template; the high molecular weight RNA served as template or primer in this reaction. About 15-30% of the total product consisted of RNA-DNA hybrids, the remainder consisting of DNA. The RNA-DNA product was a hydrogen bonded heteroduplex. An antiserum against murine mammary tumor virus (MuMTV) 5-7% of MCF-7 cells; the antiserum did not react with mouse cells infected with murine leukemia viruses (MuLV) or with non-particle-producing human cells. Absorption of the antiserum with MuMTV isopycnically banded from mouse milk markedly reduced its reactivity with MCF-7 cells. The MCF-7 particles appeared to be antigenically unrelated to MuLV.

6216 IDENTITY OF A NEWLY ISOLATED HUMAN POLYOMAVIRUS FROM A PATIENT WITH PROGRESSIVE MULTIFOCAL LEUCOENCEPHALOPATHY.

(E.) Field, A. M. (Cent. Public Health Lab., London, England), S. D. Gardner, R. A. Goodbody and M. A. Woodhouse. *J Clin Pathol* 27(5):341-347, 1974.

A new strain of polyomavirus, designated COL, was isolated from the brain of a 57-yr-old man with progressive multifocal leucoencephalopathy. Brain tissue was obtained at necropsy and explant cultures were grown and incubated. A 10% brain suspension prepared from homogenized tissue was inoculated into Vero and human lung embryo fibroblast cell cultures. Fetal brain cultures were prepared by trypsinization of brains from 10-16 week old human fetuses, and inoculated with the virus; subcultures were prepared at intervals. Virus-infected human fetal brain cell cultures were fixed in glutaraldehyde, postfixed in osmium tetroxide, and embedded in araldite-epon. Thin sections were stained with uranyl acetate and lead citrate, and examined under electron microscopy. Immune electron microscope tests were performed on the new strain of virus and five other viruses of the *Polyomavirus* genus, using appropriate antisera and the patient's serum. No cytopathic changes were evident in fetal brain cultures before two months, when moderate numbers of polyomavirus particles were seen. Approximately two weeks later definite small vacuoles appeared in the spongioblast areas: this effect could be transmitted to fresh brain cultures and was always associated with the presence of virus particles. Highly refractile single cells scattered over the cell sheet were also frequently observed. Virus particles were seen in approximately 10% of cells in human fetal brain thin sections. Round virus particles (average diameter 43.4 nm) were scattered throughout the nuclei; filamentous forms, 23.0 nm across, were occasionally seen. Round particles were also occasionally observed, usually in close association with cytoplasmic membrane. Negatively-stained particles had typical polyomavirus morphology, ranging in diameter from 38.2-56.9 nm; the larger particles were often broken open and appeared incomplete. Inoculation of Vero and human embryo lung fibroblast cultures produced no virus lines, nor did incubation from explant cultures of brain tissue. Immune electron microscope tests on the complete range of *Polyomavirus* revealed that COL is closely related antigenetically to JC human polyomavirus, but is distinct from other members of the group except SV_{40}, with which there was a slight cross reaction. It is concluded that the newly isolated COL polyoma virus is a further strain of JC virus.

6217 UPTAKE OF 2-DEOXYGLUCOSE BY BALB/3T3 CELLS: CHANGES AFTER POLYOMA INFECTION.

(E.) Eckhart, W. (Salk Inst., San Diego, Calif.) and M. Weber. *Virology* 61(1):223-228, 1974.

The rate of 2-deoxyglucose uptake in exponentially growing and resting BALB/3T3 cell cultures was examined before and after lytic infection with wild-type polyoma virus or two temperature-sensitive polyoma mutants and was compared to rates in cultures of BALB/3T3 cells transformed by simian virus 40 (SV40). The rate of uptake was approximately 3-fold faster in actively growing cells than in resting cells, and 3-fold faster in polyoma or SV40-transformed cells than in actively growing untransformed cells. The uptake of 2-deoxyglucose increased approximately

10-fold after infection of resting cells by polyoma virus and 3-fold after polyoma infection of growing cells. The uptake of 3-0-methylglucose also increased after infection of resting cells, indicating that the changes in the rate of 2-deoxyglucose uptake can be ascribed to changes in the rate of transport of hexose across the cell membrane. The increase in uptake in polyoma-infected cells began about 12 hr after the infection of resting cells at 37 C and occurred in the presence of cytosine arabinoside. The increase occurred after infection at 39 C by a polyoma temperature sensitive mutant (ts25), in which viral DNA synthesis is blocked at the restrictive temperature (39 C). The increase did not occur after infection with ts3, a mutant in which the induction of cellular DNA synthesis is blocked at the restrictive temperature (39 C); it did occur after ts3 infection at the permissive temperature (32 C). The results indicate that an early viral gene function is required to bring about the postulated change in membrane activity during lytic infection.

6218 STIMULATION OF THYMIDINE KINASE INDUCTION BY POLYOMA VIRUS IN CELLS "PRELABELED" WITH 5-BROMO-2'-DEOXYURIDINE.

(E.) Zemla, J. (Inst. Virol. Slovak Acad. Sci., Bratislava, Czechoslovakia). *Neoplasma* 21(3):359-362, 1974.

To test whether the raised activity of thymidine (dTR) kinase in mouse embryo (ME) cells infected with polyoma virus (strain SE) is of viral or cellular origin, cells were cultured in the presence of added dTR and prelabeled with 5-bromo-2'-deoxyuridine (BUDR) for 96 hr before infection. Control ME cells had dTR only added to the culture medium. The level of enzyme activity in noninfected cells was found to be similar in BUDR-treated and control cells, while in the infected cells dTR kinase activity was higher in the BUDR-treated cells. Incorporation of BUDR into DNA may provide a genetic basis for the heightened polyoma virus-induction of dTR kinase. These observations do not exclude the possibility that BUDR was active on a non-genetic level, but they indirectly support the cellular origin theory of polyoma virus-induced dTR kinase.

6219 PROPERTIES OF POLYOMA PSEUDOVIRIONS IN MOUSE AND HUMAN CELLS.

(E.) Qasba, P. K. (Univ. Maryland Sch. Med., Baltimore), D. B. Yelton, Q. A. Pletsch and H. V. Aposhian. *Collect Pap Annu Symp Fundam Cancer Res* 25:169-185, 1974.

The pseudovirions produced during infection of primary mouse embryo cells with polyoma virus (0.1 plaque-forming U/cell) were characterized. Equilibrium centrifugation in CsCl indicated that 80% or more of the particles produced during infection of tritiated thymidine-prelabeled cells were pseudovirions. These particles contained linear, double-stranded mouse DNA. Approximately 80% of the pseudoviral DNA had a sedimental value of 14S in neutral sucrose. In confluent monolayers on mouse embryo cells, 14S DNA was detected 36 hr after virus infection, production of full particles beginning at 42 hr postinfection. Experiments with secondary mouse embryo cells and with human embryonic cells demonstrated the transfer of polyoma pseudoviral DNA to these cells. The pseudoviral DNA was uncoated in such cells. Most of the DNA was protected in the cytoplasm, but some fragmentation occurred in the nucleus. That the DNA carried by pseudovirions reaches the nucleus of mouse cells suggests that pseudovirions are capable of transduction. Although the particles could also be oncogenic, no tumors have yet been detected in baby mice injected with purified pseudovirions.

6220 GRAFT VERSUS LEUKEMIA: EFFECT OF PHYTOHEMAGGLUTININ-STIMULATED AND NORMAL SPLEEN CELLS ON RAUSCHER DISEASE.

(E.) Lair, S. V. (Univ. Tennessee Mem. Res. Cent., Knoxville) and B. B. Lozzio. *J Natl Cancer Inst* 53(4):1165-1168, 1974.

The effects of normal syngeneic spleen cell grafts and grafts of spleen cells from phytohemagglutinin (PHA)-treated mice on the survival of BALB/c mice infected with Rauscher leukemia virus (RLV) was studied. Six-week-old BALB/c mice were inoculated i.p. with a cell free spleen extract containing RLV; one week later, they were inoculated i.v. with normal and PHA-stimulated spleen cells. In some cases, the mice were reinoculated with spleen cells on weeks four and eight. The animals inoculated with RLV survived longer when pretreated with a single dose of normal or PHA-stimulated spleen cells. Transfer of PHA-stimulated cells tripled the mean survival time and transfer of normal cells almost doubled the survival time compared with leukemic mice receiving no spleen cells. Three cell transfers were even more effective than one in that the animals receiving such treatment lived more than four times longer than leukemic controls. Cell viability and integrity were not required since the injection of sonically disrupted spleen cells produced similar results. The transfers apparently extended survival without affecting the degree of viremia, but more studies are needed before any conclusions about virus titer, interferon production, and antivirus antibodies in these mice can be drawn.

6221 BEHAVIOR OF THREE TYPES OF RIBOVIRUS-LIKE PARTICLES IN SEGREGATING HAMSTER X MOUSE SOMATIC HYBRIDS.

(E.) Yotsuyanagi, Y. (Cent. Mol. Genet., Gif sur-Yvette, France) and B. Ephrussi. *Proc Natl Acad Sci USA* 71(11):4575-4578, 1974.

An electron microscopic study of somatic hybrids resulting from a cross between 2*s* Syrian hamster melanoma cells harboring R-type particles and mouse fibroblasts harboring intracisternal A-type and mostly immature C-type particles was performed. During late exponential growth, cultures were double-fixed in glutaraldehyde and osmium tetroxide and embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate prior to examination. The number of viral particles of each type was counted in all cell sections encountered by systematic displacement of grids. The virus particles observed were classified on the basis of structural criteria. Inspection of the raw data thus obtained showed the distribution of R- and A-type particles among sections of any one cell line to be nonrandom owing to both inter- and intracellular heterogeneity as well as as to variability of cell size and section area. The raw data were then recalculated in terms of the average percent of sections containing at least one particle. It was found that hybrid cells that retain a large fraction of both hamster and mouse chromosomes produce all three types of particles. The loss of mouse chromosomes is followed by that of intracisternal A- and C-type particles. The continued production of A- and C-type particles is dependent on the retention of different mouse chromosomes. The mixed intracellular population of R- and A-particles comprises a small number of complex structures in which the two types of virions are enclosed with a common envelope. It is concluded that, although many types of evidence have pointed to the occurrence of hybrid or mixed particles, this is the first unambiguous electron microscopic demonstration of complex forms comprising different types of virions under a common viral coat.

6222 ALTERATIONS OF SURFACE PROPERTIES OF CHICK EMBRYO FIBROBLASTS INFECTED WITH FOUR ROUS SARCOMA VIRUSES PRODUCING DISTINCTIVE CELL TRANSFORMATION. *(E.)* Morgan, H. R. (Sch. Med. Dent., Univ. Rochester, N.Y.) and H. B. Bosmann. *Proc Soc Exp Biol Med* 146(4):1146-1149, 1974.

To determine if alterations in external membranes were similar in colonies of transformed cells with different morphologies and variations in the degree of loss of contact inhibition induced with different cloned strains of virus, Schmidt-Ruppin (RSV-SR), Bryan (RSV-Br), morph r (RSV-Br-mr), morph f (RSV-Br-mf) and Rous-associated virus (RAV-1) were used. Chick embryo primary cultures, derived from eggs of a Gs antigen-negative, leukosis-free flock, were infected with the viruses and then split on the fourth day and used on the sixth day when over 75% of the cells appeared visibly transformed. RSV-SR contained both fusiform and rounded cells with some overlapping due to loss of contact inhibition. RAV-1 was similar to the control but with more vacuoles. RSV-Br and RSV-Br-mr produced the same marked rounding of cells with piling into dense foci indicating a marked loss of contact inhibition and RSV-Br-mf produced a marked fusiform character with significant overlapping of cells but no piling into dense foci. Assays for cell surface glycosyl transferases were performed with labeled UDP galactose and GDP mannose (240 Ci/M each). The glycosyl transferase activities were elevated in all RSV strains as compared to control or RAV-1-infected cells. The electrophoretic mobilities were significantly increased in the RSV strains as compared to the control or RAV-1 cells (RSV average was -2.46, RAV and control average: -1.99 μM/sec/V/cm). It is concluded that even though the RSV strains produce distinctive types of cellular transformations, the type of morphological change or degree of loss of contact inhibition is not related to the cell surface changes (glycosyl transferase activities and electrophoretic mobilities) in RSV-transformed cells.

6223 CONTROL OF MULTIPLICATION OF NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICKEN EMBYRO FIBROBLASTS BY PURIFIED MULTIPLICATION-STIMULATING ACTIVITY WITH NONSUPPRESSIBLE INSULIN-LIKE AND SULFATION FACTOR ACTIVITIES. *(E.)* Temin, H. M. (McArdle Lab., Univ. Wisconsin, Madison), G. L. Smith and N. C. Dulak. *Cold Spring Harbor Conf Cell Proliferation* 1:19-26, 1974.

The mechanism by which the multiplication-stimulating activity (MSA) factor present in serum stimulates the multiplication of normal and Rous sarcoma virus (RSV)-transformed chicken embryo fibroblasts was studied. Using the techniques employed for the purification of nonsuppressible insulin-like activity, MSA was purified approximately 5000-fold from calf-serum. Using Dowex-50 chromatography, acetic acid-urea polyacrylamide gel electrophoresis, and SDS polyacrylamide gel electrophoresis, MSA was also purified from serum-free medium conditioned by the growth of Coon's buffalo rat liver cells (CRL). The CRL MSA was a small polypeptide with a molecular weight of about 10,000 and had no trypsin or antitrypsin activity. It was more active per mg protein than calf serum in stimulating DNA synthesis, but could apparently stimulate only half as many cells as the MSA in native calf serum. In addition, the CRL MSA had both nonsuppressible insulin-like activity and sulfation factor activity; thus it resembles somatomedin purified from human serum. Purified CRL MSA stimulated the multiplication of chicken and duck embryo fibroblasts and also supported limited multiplication of RSV-infected chicken embryo fibroblasts. RSV-infected chicken embryo fibroblasts multiplied more than uninfected fibroblasts in a medium containing 0.1% calf serum and 0.8 μg/ml CRL MSA. The stimulation of DNA synthesis by CRL MSA required amino acids and glucose in the medium and the kinetics of stimulation differed somewhat from those of calf serum. The results suggest that the mechanism of action of MSA is not to trigger an irreversible event, but to initiate and maintain a reversible process which culminates in the initiation of DNA synthesis.

6224 ALTERATIONS IN GLUCOSE METABOLISM IN CHICK EMBRYO CELLS TRANSFORMED BY ROUS SARCOMA VIRUS: TRANSFORMATION-SPECIFIC CHANGES IN THE ACTIVITIES OF KEY ENZYMES OF THE GLYCOLYTIC AND HEXOSE MONOPHOSPHATE SHUNT PATHWAYS. *(E.)* Singh, M. (Div. Biol. Sci., Albert Einstein Coll. Med., Bronx, N.Y.), V. N. Singh, J. T. August and B. L. Horecker. *Arch Biochem Biophys* 165(1):240-246, 1974.

Primary cell cultures of 10-day chick embryos were infected with either wild type Rous sarcoma virus (RSV), or the temperature-sensitive mutant (Ts-68), of the Schmidt-Ruppin strain to observe the effects of transformation on the activity of key enzymes involved in glucose metabolism. In 4-day-old secondary cultures the activities of the three rate-limiting glycolytic enzymes, hexokinase, phosphofructokinase and pyruvate kinase, as well as that of lactate dehydrogenase, were increased by about two fold. For enzymes of the hexose monophosphate shunt pathway, glucose-6-*P*-dehydrogenase activity was increased significantly while 6-*P*-gluconate dehydrogenase activity was unchanged. Hexokinase activity, in transformed as compared to normal cell fractions, was markedly increased in the particulate, i.e., bound, fraction but was unchanged in the soluble fraction. To differentiate between activity changes due to transformation, and those due to infection, the Ts-38 mutant (which is normal for infection and replication but temperature sensitive for transformation) was used. The results showed that for hexokinase, phosphofructokinase, pyruvate kinase and glucose-6-*P*-dehydrogenase increased activities and increased sugar uptake were associated specifically with the process of transformation; lactate dehydrogenase was different, i.e., infected cells had increased activity at both the permissive and nonpermissive temperatures. The increased enzyme activities of the transformed cells apparently provide a mechanism for increased flux of glycolysis and glucose transport. The increased rates of glycolysis in transformed cells appear to be genuinely related to the increase in activities of the key enzymes studied.

6225 ROUS SARCOMA VIRUS TRANSFORMATION OF THE CHICK CELL SURFACE. *(E.)* Wickus, G. G. (Dep. Biol., Massachusetts Inst. Technol., Cambridge), P. E. Branton and P. W. Robbins. *Cold Spring Harbor Conf Cell Proliferation* 1:541-546, 1974.

To determine the role of transformation-induced chemical and physical cell surface changes in the loss of normal growth control by these cells, the transformation of chick fibroblasts by Rous sarcoma virus (RSV) was studied. Secondary chick embryo fibroblasts were infected with TS-68, a temperature-sensitive mutant of the Schmidt-Ruppin strain of RSV. These cells showed normal phenotypes at 41 C and the RSV-transformed phenotypes at 36 C. The infected cells were pulse-labeled with (^{35}S)methionine immediately before and for 3 hr periods after a shift in temperature from 41 to 36 C. Two major changes occurred in the labeling pattern in association with transformation: reduction in the synthesis of a 45,000-dalton protein found in normal chick cells during the period 3-6 hr after the induction of transformation, and a rapid decrease in another protein component which was complete within the first 3 hr after the induction of transformation. The latter protein may be a cell surface protein bound loosely to the external face of the cell. Its electrophoretic properties were similar to those of the major protein of the chick cell surface, which can be iodinated by the lactoperoxidase-^{125}I system. It is possible that this component is still made after transformation but that it is hydrolyzed rapidly by transformation-specific proteolytic enzymes, however, these data do not provide evidence for or against this hypothesis.

6226 THE PREFERRED ROLE OF 60-70S AVIAN TUMOR VIRUS RNA AMONG NATURAL RNAS AS TEMPLATE FOR DNA POLYMERASE OF ROUS SARCOMA VIRUS (RSV). *(E.)* Canaani, E. (Dep. Mol. Biol., Univ. California, Berkeley) and P. H. Duesberg. *Collect Pap Annu Symp Fundam Cancer Res* 25:97-114, 1974.

Comparative analyses indicating that the 60-70S ribonucleic acid complex of avian tumor viruses has a 5- to 20-fold better template activity for Rous sarcoma virus (RSV) DNA polymerase than for other natural RNAs tested are reviewed. RNAs from different strains of avian tumor viruses work equally well with the DNA polymerase of their own virus strain as with that of other avian tumor virus strains. Heating reduces the relatively high template activity of 60-70S tumor virus RNA to the level of other natural RNAs. The midpoint of the thermal transition of the high template activity is 65 C, while the transition midpoint (Tm) of the thermal conversion of the 60-70S structure to smaller than 30-40S RNA subunits was only 50 C in 0.1 M NaCl. It was concluded that linkage among the large 30-40S subunits of the 60-70S RSV RNA complex is not the predominant reason for the high template activity of RSV RNA. Physical analysis of the primary DNA product, obtained after incubation of 60-70S RSV RNA with RSV DNA polymerase, indicated that the small subunits of the 60-70S RSV RNA complex are covalently linked to most of the DNA produced, suggesting that they serve as primers for DNA synthesis. This was confirmed by annealing experiments which showed that 60% of the high template activity of RSV RNA can be restored, after heating above its Tm, by annealing of both large (30-40S) and small (less than 10S) subunits of RSV RNA. Although the primer was not directly isolated, it appears that one or several small subunit(s) of 60-70S RSV RNA are responsible for its high template activity for the viral DNA polymerase. It is concluded that the preference of RSV DNA polymerase for 60-70S avian tumor virus is a result of the complex structure of the viral RNA which presumably provides both template and primers for *in vitro* synthesis of DNA. The relative template specificity of RSV DNA polymerase for 60-70S RSV RNA is compatible with, though not proof of, its putative role in virus replication.

6227 HOST CELL CONTROL OF THE SV40-TRANSFORMED PHENOTYPE. *(E.)* Basilico, C. (New York Univ. Sch. Med., New York, N.Y.), D. Toniolo and H. C. Renger. *Collect Pap Annu Symp Fundam Cancer Res* 25:440-457, 1974.

Host-cell control over expression of viral neoplasia was studied using temperature-sensitive (ts) SV40-transformed 3T3 cells (SV3T3). The ts SV3T3 cells are free of infectious virus, but they synthesize SV40-specific T-antigen and this function is not affected by temperature. The transformants exhibited malignant behavior *in vitro* only when growing at 32 C; at higher temperatures, they were subjected to growth control and closely resembled parental 3T3 cells. Properties of transformed cells that were temperature-dependent in the ts transformants were: (a) high saturation density, (b) ability to form colonies on 3T3 monolayers, (c) lack of contact-inhibition of DNA synthesis and (d) agglutination by wheat germ agglutinin. The property that was temperature-dependent to a small degree was serum independence. The transformants were strongly anchorage-dependent at 39 C, suggesting that these cells undergo a surface modification at 39 C which decreases their adhesiveness to one another. Reversal of transformation on a shift to 39 C required

24 hr, while retransformation on downward shift took 36-48 hr. Virus was rescuable from ts SV3T3 cells and was fully wild type with respect to infectivity or transforming ability. Thus, the ts transformants owe their behavior to a cellular rather than a viral mutation. These results demonstrate that the host cell can exert stringent control over the expression of malignancy, even when containing presumably functioning SV40 genome. Two possible mechanisms of host control are briefly considered: a repressor type of control and a ts mutation affecting ability of host protein(s) to combine with viral protein.

6228 BIOLOGICAL ANALYSIS OF CLONES OF SV40-INFECTED MOUSE 3T3 CELLS.

(E.) Risser, R. (Cold Spring Harbor Lab., N.Y.) and R. E. Pollack. *Cold Spring Harbor Conf Cell Proliferation* 1:139-150, 1974.

Clones of simian virus 40 (SV40)-infected mouse 3T3 cells were analyzed for viral antigens, growth properties in 10% and 1% calf serum, anchorage properties, colony formation on monolayers of normal cells and stability of growth properties. By indirect immunofluorescence, 25/40 clones were negative for viral capsid antigen and 20/40 were negative for SV40-specific T antigen. T antigen levels measured by complement fixation were similar to those found with immunofluorescence. In 10% serum, uniformly T antigen-positive clones had high saturation densities and clones with an intermediate staining pattern had intermediate densities; antigen-negative clones gave low values closer to those of 3T3 cells. Doubling time in 1% serum did not correlate with the T antigen level of a given clone. In methylcellulose, T antigen-positive clones plated with an efficiency of 10-50% compared with an efficiency of 0.5-10% for the intermediate clones. Although capable of a number of divisions in methylcellulose, cells from clones with intermediate T antigen did not form a visible colony in the three-week assay time. While most uniformly T antigen-positive clones plated with equal efficiency on plastic or on 3T3 monolayers, intermediate clones varied widely in their plating efficiency on 3T3 monolayers as compared with their plating efficiency on plastic. In general, monolayer plating correlated with the saturation density of an intermediate clone. Antigen-negative clones did not form visible colonies on normal monolayers. Growth characteristics of the three types of SV40-transformed clones remained stable after five months of continuous passage and recloning. In no case was a T antigen-negative subclone generated from a T antigen intermediate clone. The data demonstrate that the effect of SV40 on 3T3 cells is more varied and extensive than previously assumed.

6229 DEFECTIVE SIMIAN VIRUS 40 GENOMES: ISOLATION AND GROWTH OF INDIVIDUAL CLONES.

(E.) Mertz, J. E. (Stanford Univ. Med. Cent., California) and P. Berg. *Virology* 62(1):112-124, 1974.

A general method for cloning, propagating, and assaying the infectivity of defective simian virus 40 (SV40) mutants having gross alterations in the structure of their genomes is described. Eleven defective deletion mutants were isolated by this method which uses complementation with temperature-sensitive mutants as helpers. Procedures for screening for the presence of mutants, plaque-purifying, propagating, assaying the titer of stocks of these mutants, and assigning mutants to complementation groups are included. The starting material for the isolation of mutants was obtained by four serial passages of wild-type SV40 on primary African green monkey kidney cells using the undiluted lysate obtained from each passage to initiate the subsequent infection. Of the DNA molecules in this stock, 55% were resistant to cleavage by *Eco*RI endonuclease, 47% were resistant to HpaII endonuclease (a restriction nuclease from *Hemophilus parainfluenzae)*, and 23% were resistant to both enzymes. The *Eco*RI endonuclease-resistant DNA molecules were each separated from the rest of the molecules and these two heterogeneous DNA preparations served as the sources of defective SV40 DNA. Restriction endonucleases were used to select from the defective DNA preparations for mutant molecules that lacked the corresponding restriction sequence; this same property was used to separate the cloned defective mutants from their complementing helper DNA. Electron microscopic examination of each of the restriction endonuclease-resistant DNAs revealed an essentially homogeneous population of circular molecules, the mean sizes of the various mutants ranging from 0.71 to 0.96 SV40 fractional length. Thus, this procedure permits isolation of individual clones of defective mutants of SV40. It is suggested that these same procedures may be adapted to permit isolation of deletion mutants of other animal viruses.

6230 HOST CELL CONTROL OF VIRAL TRANSFORMATION.

(E.) Basilico, C. (New York Univ. Sch. Med., N.Y.), H. C. Renger, S. J. Burstin and D. Toniolo. *Cold Spring Harbor Conf Cell Proliferation* 1:167-176, 1974.

To determine how viral and cellular functions control the expression of cellular transformation, the possible mechanisms underlying the temperature-sensitive (ts) expression of the transformed phenotype by a ts SV40-transformed 3T3 cell line (ts SV 3T3) were studied. These cells express the transformed phenotype at 32 C but not at 39 C and they represent mutations in a host gene(s) which controls the expression of malignant transformation. At the permissive temperature, the ts SV3T3 cells reached high saturation densities, formed colonies when plated in 3T3 monolayers, showed no density inhibition of DNA synthesis, and showed enhanced agglutination by plant lectins. The ts transformants were free of infectious virus, synthesized SV40-specific T antigen at all temperatures, and apparently owed their behavior to a cellular, rather than a viral, transformation. Variations in total intracellular cyclic AMP did not appear to play a major role in determining the different behavior of the ts SV3T3 cells at permissive and nonpermissive temperatures. Although the serum requirements of the ts SV3T3 cells were higher at 39 C than at 32 C, the relationship between serum requirements and the other properties of transformed cells remains unresolved. Although attempts to retransform ts SV3T3 cells with high multiplicities of SV40 at 39 C were unsuccessful, these cells were fully susceptible to retransformation with murine sarcoma virus at this temperature. Infection with polyoma virus or SV40 at high m.o.i. (200 PFU/cell) was capable of inducing cellular DNA synthesis in a large proportion of the cells at 39 C. The results indicate that the host cell can exert control over the expression of SV40-induced transformation and that this control is probably at the level of a regulatory mechanism. The data also suggest the involvement of a host cell protein.

6231 CELL CYCLE DEPENDENT MODULATIONS OF THE SURFACE MEMBRANE OF NORMAL AND SV40 VIRUS TRANSFORMED 3T3 CELLS. *(E.)* Smets, L. A. (Netherlands Cancer Inst., Amsterdam) and L. De Ley. *J Cell Physiol* 84(3):343-348, 1974.

Agglutinability with Concanavalin A was studied as a function of cell cycle transition in normal and SV40 virus-transformed mouse 3T3 fibroblasts. In synchronized cultures of normal cells, agglutinability was high during mitosis and disappeared rapidly. Agglutinability of transformed cells remained high in G_1 phase but diminished gradually upon entering S phase and reached a minimum in G_2 phase. Decreased agglutinability at the end of the cell cycle was also observed in asynchronous SV3T3 cultures by a combined technique of hemadsorption and density gradient centrifugation. In normal 3T3 cells, similar variations in agglutinability during interphase could not be observed. It is suggested that the previously published concept that transformed cells remain frozen or blocked in their mitotic membrane configuration should be refined (at least for the agglutination with Concanavalin A of SV3T3 cells) since agglutination is modulated in the course of the cell cycle. The first modulation occurs when mitotic cells enter G_1 phase and the second when the SV3T3 cells enter S phase, resulting in a six- to seven-fold reduction of agglutinability. It is further suggested that modulation of agglutinability during interphase could be a specific phenomenon of transformed cells since it was not observed in 3T3 cells.

6232 CHARACTERIZATION OF A REARRANGEMENT IN VIRAL DNA: MAPPING OF THE CIRCULAR SIMIAN VIRUS 40-LIKE DNA CONTAINING A TRIPLICATION OF A SPECIFIC ONE-THIRD OF THE VIRAL GENOME. *(E.)* Koury, G. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.), G. C. Fareed, K. Berry and M. A. Martin. *J Mol Biol* 87(1):289-301, 1974.

The results of experiments, designed to map the triplicated genome segment of a simian virus 40-like virus (DAR), are presented. DAR-heavy virions, isolated from human brain, were purified and passaged serially in primary African green monkey kidney cells. Results indicated that simian virus 40 complementary RNA transcribed *in vitro* with *Escherichia coli* RNA polymerase from one strand of simian virus 40 DNA reacted with both strands of denatured 10.4 S cleavage product. The treatment of the 10.4 S DNA-simian virus 40 complementary RNA hybrid, with a single strand specific nuclease, converted about half of the radioactive counts to an acid-soluble product. The 10.4 S product contained a transposition of sequences, present originally on one of the DAR DNA strands, to the other strand. A molecular rearrangement(s) may result in the recombination and inversion of viral DNA sequences from two loci on the parental DAR genome. This 1.1 x 10^5 dalton segment is reiterated in a supercoiled molecule three times.

6233 CHANGES OF REGULATION OF HOST DNA SYNTHESIS AND VIRAL DNA INTEGRATION IN SV40-INFECTED CELLS. *(E.)* Hirai, K. (Wistar Inst. Anat. Biol., Philadelphia, Pa.), G. Campbell and V. Defendi. *Cold Spring Harbor Conf Cell Proliferation* 1:151-165, 1974.

Previous studies on simian virus 40 (SV40)-infected Chinese hamster embryo cultures demonstrated an alteration in control of DNA synthesis that was expressed in the appearance of polyploidy. The biological significance of polyploid cells was examined by monitoring (1) the fate of the viral genome and its relationship to host DNA, and (2) the degree of polyploidy in relation to the appearance of the transformed state. In SV40-infected Chinese hamster cells, induction of cell DNA synthesis and viral DNA integration showed a good concurrence in terms of order of appearance, dependence on the functioning viral genome and on culture conditions. However, viral DNA was integrated even when induction of cell DNA synthesis was abolished by drugs or by use of a lethal virus mutant. Thus, integration of viral DNA does not depend on replication of cell DNA and precedes, at least functionally, the induction of cell DNA synthesis. Reinitiation of cell DNA synthesis was demonstrated by the appearance of tetraploid metaphases at the time of first mitosis after infection and by the finding of heavy-heavy (HH) DNA when normal cycling through mitosis was inhibited. The total amount and the percentage of HH DNA (newly synthesized cell DNA) synthesized during the first 48 hr postinfection was directly proportional to the amount of integrated viral DNA. Chromosome analyses were performed on transformed colonies selected by the agar method and on transformed colonies selected by the morphological phenotype in monolayers. In both groups of colonies, the distribution of tetraploid frequency indicated that transformed colonies do not originate exclusively from infected, preexisting tetraploid cells. At a time when 30% of cells were T antigen-positive and 20% of metaphases were polyploid, an infected population was fractionated in an albumen gradient that separated cells on the basis of volume. Both polyploid cells and cells capable of producing transformed colonies were concentrated in the first two pooled fractions, which contained only a minority of the total cell population. Therefore, cell populations containing more tetraploid cells originate more transformed colonies than those containing few tetraploid cells. These results suggest that reinitiation of DNA synthesis may result from viral DNA integration, and that polyploid cells are the source of the transformed population.

6234 TWO CLASSES OF REVERTANTS ISOLATED FROM SV40-TRANSFORMED 3T3 MOUSE CELLS. *(E.)*

Vogel, A. (Cold Spring Harbor Lab., N.Y.), J. Oey and R. Pollack. *Cold Spring Harbor Conf Cell Proliferation* 1:125-138, 1974.

To determine which of the selective and nonselective differences between 3T3 and SV40-transformed 3T3 cells (SV101) are the consequence of the continued expression of integrated viral genes, sublines which have reverted to resemble 3T3 in some or all of these differences were isolated, characterized and compared with the transformants with respect to SV40 viral expression. Two classes of revertants, serum and density revertants, were isolated. While it was possible to affect the saturation density of the cultured cells without altering their serum requirements, it was not possible to alter the serum requirements without simultaneously altering the saturation density. A virus-transformed line with a high saturation density and normal serum requirements has not yet been isolated. Normal 3T3 cells and the serum revertants were unable to grow in 1% calf serum and had very high cAMP levels under these conditions, whereas the SV101 transformant and the density revertants were able to grow in 1% calf serum and displayed similar low cAMP levels under these conditions. In addition, the density revertant, F1SV101, showed a density-dependent decrease in the rate of DNA synthesis, in the fraction of cells synthesizing DNA and in the fraction of cells in mitosis. Although these cells did respond to cellular contact, the shut-off of DNA synthesis was not as "tight" as in confluent normal 3T3 cells. It is not clear how the serum revertants maintained a low saturation density in 10% calf serum. The revertants all contained SV40 T antigen, the amount and strand specificity of the SV40-specific RNA in these cells resembling those of the transformed parent. Thus, the revertant phenotypes were not the result of an inability to transcribe the SV40 genome. The fact that nondefective SV40 could be rescued from the revertants implies that the reversion in transformed growth properties does not require all copies of any single SV40 gene in the cell to be defective.

6235 STRUCTURE OF TWO ADENOVIRUS-SIMIAN VIRUS 40 HYBRIDS WHICH CONTAIN THE ENTIRE SV40 GENOME. *(E.)*

Kelly, T. J., Jr. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.), A. M. Lewis, Jr., A. S. Levine and S. Siegel. *J Mol Biol* 89(1):113-126, 1974.

A study was made of two defective adenovirus-simian virus 40 hybrids that contain the entire SV40 genome (Ad2^{++}HEY and Ad2^{++}LEY). SV40 virus (small plaque, strain 776) was grown on monolayers of BSC-1 cells. Results indicated that both hybrids gave rise to infectious SV40 virions upon the infection of cells that were permissive for SV40. However, the efficiencies were markedly different. In the case of Ad2^{++}LEY, infectious SV40 was produced in one in 10^4 cells infected. However, in the case of Ad2^{++}HEY, most of the cells that were infected with a hybrid particle yielded SV40 progeny. Ad2^{++}LEY contained two hybrids, while Ad2^{++}HEY contained three hybrids. The content of SV40 DNA of the Ad2^{++}HEY varied from 0.03-1.05 SV40 genomes, while the content of SV40 DNA of the Ad2^{++}LEY varied from 0.45-2.39 SV40 genomes. It is hypothesized that since the SV40 DNA segments of HEY-II and HEY-III contain longer tandem repetitions, they yield infectious SV40 with greater efficiency than LEY-II.

6236 PERSISTENCE OF LATE SV40 GENOME TRANSCRIPTION AFTER INHIBITION OF DNA REPLICATION BY CYTOSINE ARABINOSIDE. *(E.)*

Brander, G. (Dep. Virol., Hyg., Univ. Freiburg, West Germany) and N. Mueller. *FEBS Lett* 42(2):124-126, 1974.

The effect of cytosine arabinoside (ara-C, 40 μg/ml) on late simian virus 40 (SV40) genome transcription after onset of SV40 DNA replication was studied in monolayers of African green monkey kidney cells infected with 10 plaque-forming U/cell. Rates of inhibition of ^{3}H-thymidine incorporation confirmed that late SV40 transcription was carried out after inhibition of host and viral DNA replication by ara-C. Cultures were labeled with ^{3}H-uridine for 2 hr following interruption of DNA replication and the SV40-specific RNA content of the cultures was determined by filter hybridization with SV40 DNA. The rate of SV40 transcription was unaffected for some hours, then gradually decreased. Competition hybridization experiments revealed that both unlabeled late lytic SV40 RNA and unlabeled early SV40-specific RNA could compete with ^{3}H-labeled SV40-specific RNA (90-95% and 40% competition, resp.). Control experiments showed extensive competition (95%) between unlabeled early SV40 RNA and labeled early SV40 RNA. These findings indicate that interruption of SV40 DNA replication by ara-C does not impair either the viral transcription rate or the transcription of late virus-specific sequences if the interruption occurs after initiation of DNA replication.

6237 INTEGRATION OF PROGENY SIMIAN VIRUS 40 DNA INTO THE HOST CELL GENOME. *(E.)*

Holzel, F. (Wistar Inst. Anat. Biol., Philadelphia, Pa.) and F. Sokol. *J Mol Biol* 84(1):423-444, 1974.

A study was made, by means of nucleic acid hybridization techniques, of progeny simian viral 40 DNA integration into the host cell DNA. Purification included precipitation of cellular DNA, velocity centrifugation (in alkaline sucrose gradients), and equilibrium centrifugation (in ethidium bromide/CsCl solution). The purified cellular DNA, which was isolated from the cells late after infection, contained 0.006% free viral DNA. However, this cellular DNA contained about 2% integrated simian virus 40 DNA. As determined by DNA-DNA reassociation kinetics, this corresponds to approximately 20,000 integrated virus genome equivalents/cell. One day after infection, integration of the simian virus 40 DNA into cellular DNA was detected. This integration increased with the increase in the rate of synthesis of viral DNA. Replacement of 2% of host-specific by virus-specific nucleotide sequences in the cellular DNA of productively infected cells may cause alterations in the regulation of transcription and replication of the host cell genome that eventually result in cell death.

6238 ULTRASTRUCTURAL LOCALIZATION OF CONCANAVALIN A RECEPTORS IN SOMATIC CELL HYBRIDS BETWEEN NORMAL MOUSE CELLS AND SIMIAN VIRUS 40-TRANSFORMED RAT CELLS. *(E.)* Walig, C. (Lab. Gezond., Univ. Amsterdam, Holland), J. M. M. Walboomers and J. van der Noordaa. *J Cell Biol* 61(2):553-557, 1974.

The staining properties of concanavalin A-peroxidase on normal 3T3 TK mouse cells, normal BN/BI rat cells, Simian Virus 40-transformed rat brain cells, and a somatic cell hybrid between normal mouse cells and Simian Virus 40-transformed rat cells were studied. Cell cultures were incubated for 15 min in a solution of concanavalin A (50 μg/ml in phosphate-buffered saline), washed, incubated for 15 min in a solution of peroxidase (50 μg/ml in phosphate-buffered saline), washed again and fixed for 15 min in glutaraldehyde solution (1.6% in phosphate-buffered saline). A continuous staining pattern formed on normal and transformed cells which was impossible to differentiate. The concanavalin A-peroxidase method of staining does not seem suitable for the study of Simian Virus 40 genome expression since receptor sites on the parent cells and hybrid line could not be distinguished.

6239 ISOLATION AND CHARACTERIZATION OF TWO CLASSES OF ConA-RESISTANT SV3T3 CELLS. *(E.)* Ozanne, B. (Cold Spring Harbor Lab., N.Y.) and M. Lurye. *Cold Spring Harbor Conf Cell Proliferation* 1:177-190, 1974.

The characteristics of several previously reported Concanavalin A (ConA)-resistant Simian Virus 40 (SV40)-transformed 3T3 cells (SV3T3) were studied, along with those of a newly isolated class of ConA-resistant SV3T3 cells. Two classes of ConA-resistant SV3T3 cells were obtained: those which failed to form multilayers of cells and had low saturation densities and those which consistently overgrew the monolayer and grew to high saturation densities. Both groups were able to grow in 1% serum, continued to synthesize DNA after reaching saturation density, failed to form colonies when grown in Methocel, had the same cyclic AMP levels as normal 3T3 cells, failed to agglutinate at high concentrations of ConA or WGA, became agglutinable following exposure to trypsin, were resistant to the toxic effects of ConA, and bound approximately the same number of radioactive ConA molecules. While the revertants contained a slightly increased number of chromosomes per cell, they contained almost twice as much DNA per cell as their parental SV3T3 cells. All of the cells of the revertant lines were positive for the presence of the SV40-specific T antigen, and infectious wild-type SV40 could be rescued from all lines. However, the revertants were resistant to retransformation by both homologous and heterologous viruses. The results strongly suggest that an altered cellular function, rather than a deficiency in a viral function, was responsible for the revertant phenotype.

6240 LACK OF CORRELATION BETWEEN FRIBRINOLYSIS AND THE TRANSFORMED STATE OF CULTURED MAMMALIAN CELLS. *(E.)* Mott, D. M. (Fox Chase Cancer Cent., Philadelphia, Pa.), P. H. Fabish, B. P. Sani and S. Sorof. *Biochem Biophys Res Commun* 61(2):571-577, 1974.

Ten different types of serially cultured, growing cells were examined for their extracellular fibrinolytic activity in relation to their transformed or non-transformed states. The cells examined were 1) Swiss mouse fibroblasts 3T3 2) their SV-40 virus-transformed 3T3 derivatives 3) spontaneously transformed Chinese hamster ovary cells (CHO-K1) 4) Chinese hamster kidney fibroblasts (CHK-C16a) 5) an SV-40 transformed subline of CHK-C16a 6) a polyoma virus transformed subline of CHK-C16a 7) human diploid non-transformed cells (WI-38) 8) human diploid nontransformed cells (WI-26) 9) SV-40 virus-transformed WI-38 cells and 10) SV-40 virus-transformed WI-26 cells. Subconfluent monolayer cultures of the cells were maintained in serum-free medium for 18 hr, after which extracellular medium was removed and centrifuged to yield cell-free harvest medium. Densities were then 1.0 - 4.8 x 10^4 cells/cm^2. Two ml of harvest medium and 61 μg purified human plasminogen were added to ^{125}I-fibrin-coated 35 mm dishes, incubated for 18 hr at 37 C in 5% CO_2 atomsphere, and the ^{125}I in the supernatant digest fluids was counted. The concentration of plasminogen activator in harvest medium was compared on the basis of activator liberated by 10^5 cells/5 cm^2/ml medium. The nontransformed CHK cells and their SV-40 virus-transformed subline liberated little and no detected activator, resp. Both the control 3T3 cells and the transformed SV-3T3 cells produced high levels of extracellular activator activity. Both kinds of human diploid nontransformed fibroblasts exhibited rather high activator activities. Their SV-40 transformed derivative cells produced virtually no detected activator. The spontaneously transformed CHO fibroblasts liberated a high level of plasminogen activator. It is concluded that the levels of extracellular fibrinolytic activity exhibited *in vitro* by extensively cultured growing cells do not correlate with their state of transformation or nontransformation.

6241 OCCURRENCE OF C-TYPE VIRUSES IN BABOON (*PAPIO CYNOCEPHALUS*) PREIMPLANTATION EMBRYOS. *(E.)* Kalter, S. S. (Microbiol. Infect. Dis., Southwest Found. Res. Educ., San Antonio, Tex.), R. J. Helmke, G. C. Smith and R. L. Heberling. *Fed Proc* 33(3):753, 1974.

6242 ONCORNAVIRUS-LIKE PARTICLES IN NORMAL HUMAN MAMMARY CELLS IN CULTURE. *(E.)* Furmanski, P. (Michigan Cancer Found., Detroit), C. Longley, D. Fouchey, R. Rich and M. A. Rich. *Fed Proc* 33(3):753, 1974.

6243 CHARACTERIZATION OF ONCORNAVIRUS ISOLATED FROM NZB LYMPHOBLASTOID CELLS. I. HOST RANGE AND LEUKEMOGENIC POTENTIAL. *(E.)* Del Villano, B. C. (Scripps Clin. Res. Found., La Jolla, Calif.), B. P. Croker and F. J. Dixon. *Fed Proc* 33(3):753, 1974.

6244 POSITIVE CONTROL OF THE TRANSFORMED PHENOTYPE IN HUMAN HYBRIDS BETWEEN OLD NORMAL FIBROBLASTS AND SV40 TRANSFORMED CELLS. *(E.)* Green, C. (Wistar Inst., Philadelphia, Pa.), H. Koprowski and C. Croce. *Fed Proc* 33(3):729, 1974.

6245 PROSTAGLANDIN CONTENT OF NORMAL AND SIMIAN VIRUS 40 TRANSFORMED BALB 3T3 CELLS. *(E.)* Ritzi, E. M. (Worcester Found. Exp. Biol., Shrewsbury, Mass.) and W. A. Stylos. *Fed Proc* 33(3):730, 1974.

6246 GLUTAMINE AVAILABILITY: A CRITICAL FACTOR FOR PERMISSIVENESS OF THE SHOPE PAPILLOMA VIRUS IN RABBITS. *(E.)* Rogers, S. (Dep. Biochem., Univ. Tennessee, Memphis) and S. El Khatib. *Fed Proc* 33(3):729, 1974.

6247 TRANSFER RNA SYNTHESIS IN ADENOVIRUS-INFECTED CELLS. *(E.)* Raska, K. Jr. (Rutgers Med. Sch., Piscataway, N.J.) and F. Varricchio. *Fed Proc* 33(3):635, 1974.

6248 HORIZONTAL TRANSMISSION OF GROSS LEUKEMIA BY PRELEUKEMIC SKIN TRANSPLANTATION. *(E.)* Mariani, T. (Pathol. Lab. Med., Univ. Minnesota, Minneapolis). *Fed Proc* 33(3):602, 1974.

6249 CHARACTERIZATION OF AN ENDOGENOUS RNA TUMOR VIRUS FROM NORMAL RAT CELLS. *(E.)* Schwartz, S. A. (Dep. Pathol., Univ. Chicago, Ill.) and W. H. Kirsten. *Fed Proc* 33(3):602, 1974.

6250 SEX DIFFERENCES IN THE INDUCTION, PROGRESSION AND LETHALITY OF MOLONEY SARCOMA VIRUS (MSV) TUMORS. *(E.)* Krzych, U. (Dep. Zool. Physiol., Rutgers Univ., Newark, N.J.), J. L. Humes and H. R. Strausser. *Fed Proc* 33(3):602, 1974.

6251 ALTERATIONS IN CYCLIC AMP METABOLISM AND MITOGEN-INDUCED PROLIFERATION OF THYMUS CELLS DURING AKR LEUKEMOGENESIS. *(E.)* Duquesnoy, R. J. (Med. Coll. Wisconsin, Milwaukee), R. G. Kemp and G. M. Pedersen. *Fed Proc* 33(3):597, 1974.

6252 RADIOACTIVE STRONTIUM (89Sr) ABROGATES GENETIC RESISTANCE TO FRIEND VIRUS LEUKEMIA. *(E.)* Kumar, V. (Boston Univ. Sch. Med., Mass.) and M. Bennett. *Fed Proc* 33(3):602, 1974.

6253 CHARACTERIZATION OF THE LEUKOVIRUS-LIKE PARTICLE IN CULTURES OF HUMAN LEUKEMIC MARROWS FROM PATIENTS IN RELAPSE AND REMISSION. *(E.)* Mak, T. W. (Dep. Med., Univ. Toronto, Canada), J. Manaster, D. E. Housman, J. E. Till and E. A. McCulloch. *Fed Proc* 33(3):602, 1974.

6254 ELECTROPHORETIC LIGHT SCATTERING (ELS) OF RAUSCHER MURINE LEUKEMIA VIRUS s. *(E.)* Gill, D. (Sci. Res. Lab., Ford Motor Co., Detroit, Mich.), L. Rimai, I. T. Salmeen, L. Liebes, M. A. Rich and J. J. McCormick. *Fed Proc [II]* 33(5):1603, 1974.

6255 THE INFIDELITY OF AVIAN MYELOBLASTOSIS VIRUS DNA POLYMERASE IN POLYNUCLEOTIDE REPLICATION. *(E.)* Battula, N. (Univ. Pennsylvania, Philadelphia). *Diss Abstr Int [B]* 35(4):1761-1762, 1974.

6256 A STUDY OF AVIAN ONCORNAVIRUS TRANSFER RNA. *(E.)* Elder, C. T. (Univ. Colorado, Boulder). *Diss Abstr Int [B]* 35(4):1539-1540, 1974.

6257 RESCUE OF SV40 FROM SV40-TRANSFORMED CELLS. *(E.)* Huebner, F. K. (Univ. Pennsylvania, Philadelphia). *Diss Abstr Int [B]* 35(4):1810-1811, 1974.

6258 REPLICATION OF EPSTEIN-BARR VIRUS IN HUMAN-PRIMATE HYBRID CELLS. *(E.)* Tsang, K. (Bowling Green State Univ., Ohio). *Diss Abstr Int [B]* 35(4):1817, 1974.

6259 DIFFERENCES IN MEMBRANE FLUIDITY BETWEEN NORMAL AND TRANSFORMED CELLS. *(E.)* Barnett, R. E. (Univ. Minnesota, Minneapolis), R. E. Scott, L. T. Furcht, J. H. Kersey, N. D. Goldberg and J. R. Sheppard. *Fed Proc [II]* 33(5):1415, 1974.

6260 STUDIES ON C4 INACTIVATOR INDUCED BY ADENOVIRUSES IN KB CELL CULTURES. *(E.)* Kosa, Z. (Natl. Inst. Publ. Health, Budapest, Hungary) and G. Fust. *Acta Microbiol Acad Sci Hung* 20(4):283-289, 1973.

6261 ISOLATION OF CELL SURFACE GLYCOPROTEINS FROM NORMAL AND TRANSFORMED CELLS BY AFFINITY CHROMATOGRAPHY ON PLANT LECTIN COLUMNS. *(E.)* Smart, J. E. (Imp. Cancer Res. Fund, London, England), E. Pearlstein and M. D. Waterfield. *Biochem Soc Trans* 2(6):1316-1317, 1974.

See also:

* (Rev): 6014, 6015, 6016, 6017, 6018, 6019, 6020, 6021, 6022, 6023, 6024, 6025, 6026, 6027, 6028, 6031, 6052, 6053, 6064, 6075
* (Immun): 6263, 6264, 6265, 6275, 6277, 6287, 6288, 6294, 6296, 6301, 6317, 6318, 6321, 6325, 6349, 6350, 6352, 6359, 6362
* (Epid-Biom): 6443

6262 IMMUNOCOMPETENCE IN ADVANCED CANCER PATIENTS PRIOR TO CHEMOTHERAPY. *(E.)*

Lehane, D. E. (Baylor Coll. Med., Houston, Tex.) and M. Lane. *Oncology* 30(6):458-466, 1974.

An evaluation was made of immunocompetence in 30 patients with advanced head and neck and lung cancer using a broad panel of immunologic tests that measured established and primary immunologic reactivity in both the antibody and cell-mediated arms of the immune system. Serum immunoglobulin levels and complement levels were measured by the radial immunodiffusion technique. Results showed that, when compared to controls, cell-mediated immunity parameters were decreased significantly in the 30 patients. The antibody-mediated immunity tests indicated significantly increased levels of serum IgA and complement and decreased levels of isoantibody titers to blood group antigens. As compared to controls, there was no difference in titrations for established virus antibodies. Following primary vaccination with keyhold limpet hemocyanin, the patients showed defects in the cell-mediated and antibody immune response. The patients with advanced lung and head and neck cancers may require immunologic intervention to augment their apparent immunoincompetence.

6263 IMMUNOLOGIC ACTIVATION OF MURINE LEUKEMIA VIRUSES. *(E.)*

Melief, C. J. M. (Dep. Hematol., New England Med. Ctr. Hosp., Boston, Mass.), S. Datta, S. Louie and R. S. Schwartz. *Cancer* 34(4): 1481-1487, 1974.

Neoplasms of lymphoid tissue apparently arise, not as a result of immunosuppression, but as a consequence of immunostimulation. The concept of immunoregulation by an input/output mechanism leads to the identification of three circumstances of faulty control: absence of antigen, excess of antigen, and insufficiency of antibody or suppressor cells. Inoculation of spleen cells from highly inbreds parental lines into Fl hybrids is followed by a graft *versus* host reaction (GVHR) in mice which causes a malignant lymphoma due to immune factors, including autoimmune complications. In this sytem, a complication is that oncornaviruses which can be oncogenic are found in the host's proliferating lymphoid tissue. Donor-type neoplasms arise when donor cells are free to proliferate in the absence of allogeneic inhibition and host-type lymphomas arise when the proliferation of donor cells is suppressed by allogeneic inhibition. Infectious C-type RNA viruses, found in the spleens of mice undergoing the GVHR, are oncogenic in BALB/c X A/J mice. Oncornaviruses have been immunologically activated only by transplantation reactions: the GVHR, the MLR (the *in vitro* analog of the GVHR), and in the spleens of mice rejecting skin allografts. Thus, lymphocyte activation caused by histoincompatibility activates murine leukemia viruses while other stimulants of lymphocytes do not. In particular, activation of murine leukemia by the MLR and GVHR may hinge on the products of the genes of the H-2 complex, although the entry and replication of oncornaviruses does not seem to require an H-2-related transformation of the lymphocyte. Other test systems indicate that cytotoxic cells, rather than those that simply transform into lymphoblasts, are involved in the activation of oncornaviruses. Lymphocytes of normal mice are capable of engaging in all of the known sequences of the life cycle of C type RNA viruses. Perhaps the lack of immunoregulatory lymphocytes, rather than the viruses, are the ultimate cause of malignancy.

6264 IMMUNOLOGIC ACTIVATION OF ONCOGENIC VIRUSES. *(E.)*

Phillips, S. M. (Div. Commun. Dis. Immunol., Walter Reed Army Inst. Res., Washington, D.C.). *Prog Exp Tumor Res* 19:37-44, 1974.

The relationship between immunologic stimulation of lymphoid cells (e.g. graft-versus-host reaction or allogeneic stimulation) and activation of latent, carcinogenic viruses in mice was studied. Cultures of spleen cells from uninoculated BALB/c x A/J F_1 (C/A F_1) mice previously inoculated with parental strain BALB/c cells were positive for leukemia virus in 10/11 experiments. Three to seven day old cultures of BALB/c and C/A F_1 spleen cells were positive for leukemia viruses by the mixed culture assay in 7/11 experiments. In three cultures that did not produce virus, lymphocyte stimulation was suboptimal, with isotope incorporation ratios only 2.5 times those of the controls, whereas the seven positive cultures were stimulated a minimum of five times the controls. C/A F_1 and BALB/c spleen cells cultured separately for the same periods were negative for virus. In addition, cells from stimulated animals lost their responsiveness to such *in vitro* transforming agents as phytohemagglutinin and concanavalin A. When normal cells were co-cultured with *in vivo* stimulated cells, they too lost their responsiveness. *In vivo* (graft) stimulation gave the highest incidence of viral activation but only *in vitro* stimulation by the mixed lymphocyte reaction or IUDR treatment resulted in the release of viral particles. The variety of stimulation factors demonstrated however, makes the possible mechanism for viral activation very complex particularly when it appears that the feedback mechanism of viral release is itself immunosuppressive and thereby reinforces the activation of oncogenic virus.

6265 AUGMENTED IMMUNOGENICITY OF TUMOR CELL HOMOGENATES INFECTED WITH INFLUENZA VIRUS. *(E.)*

Boone, C. W. (Natl. Cancer Inst., Bethesda, Md.). *Recent Results Cancer Res* 47:394-400, 1974.

The use of influenza virus to augment the immunogenicity of tumor-transplantation antigens (TTA) from tumor homogenates was studied. A transplantable simian virus 40-induced fibrosarcoma in BALB/c mice infected with WSA strain mouse-adapted influenza virus was used. Homogenates of sarcoma cells infected with influenza virus and tested for TTA activity showed tumor proliferation to be reduced. Specific virus infection of tumor cells was seen. A simple non-specific stimulation of the immune system was shown not to be the method of TTA enhancing by the virus. The length of time required to perform the skin graft rejection TTA assay prompted development of the radioisotopic footpad assay, in which the eliciting dose of tumor cells is injected into the footpad of a tumor-immune mouse, and radioiodine-labeled serum protein is given i.p. Twenty-four hours later both feet are cut off and counted using a gamma spectrometer. The ratio of counts in the test foot to that of the contralateral control foot is taken to be a measure of the intensity of the reaction. When tested in conjunction with the tumor transplant rejection assay, results indicated the footpad assay to be more sensitive and able to detect amounts of TTA insufficient to immunize against tumor challenge. The author suggests that influenza-virus antigens and the viability-independent TTA together induce the immune response against the TTA alone.

6266 A PRELIMINARY INVESTIGATION INTO THE ROLE OF IMMUNITY IN MODIFYING THE BLOOD-BORNE SPREAD OF CHEMICALLY INDUCED RAT SARCOMAS.

(E.) Proctor, J. W. (Div. Tumour Immunol., Chester Beatty Res. Inst., Sutton, England), C.-M. Rudenstam and P. Alexander. *J Natl Cancer Inst* 53(6):1671-1676, 1974.

3-Methylcholanthrene-induced (MC-1 and MC-3) and dibenzpyrene-induced (HSH and A) tumors and an ethionine-induced hepatoma were prepared as suspensions and injected i.m. into inbred male Chester Beatty Hooded rats. MC-1 and A did not metastasize spontaneously after i.m. implantation, whereas HSH, MC-3, and the hepatoma metastasized spontaneously to the lung in 10-100% of injected animals. Other rats were immunized with irradiated tumor cells, then challenged i.m. with enzyme suspensions of live tumor cells. This procedure provided strong protection against the nonmetastatisizing tumors but was ineffective against those producing high incidences of metastases. Alternatively, immunity evoked by a growing tumor in the hind leg (concomitant immunity) was demonstrated by both metastasizing and nonmetastasizing tumors, although with the latter the immunity produced was weaker than that produced by inoculation of irradiated tumor cells. Concomitant immunity to a metastasizing tumor was expressed more strongly against cells injected i.v. or intradermally than against those injected i.m. The failure of a metastasizing tumor to immunize after irradiation is interpreted in terms of the spontaneous shedding of antigens. This hypothesis suggests a link between the capacity of sarcoma cells to metastasize spontaneously and the destruction by irradiation of the capacity of such cells to immunize.

6267 CELLULAR IMMUNOABSORBENTS: A NEW APPROACH IN TRANSPLANTATION AND TUMOR IMMUNOLOGY.

(E.) Berke, G. (Dep. Cell Biol., Weizmann Inst. Sci., Rehovot, Israel) and R. D. Stulting. *Isr J Med Sci* 10(8):992-1000, 1974.

The binding of immune and normal lymphocytes to arget cells was studied using cellular immunoabsorbents. It was found that lymphocytes reactive *in vitro* and *in vivo* could be depleted from sensitized lymphoid cell populations on the basis of their ability to bind to fibroblast or polylysine-fixed tumor cell monolayers. For example, ^{37}Cr-labeled peritoneal exudate lymphocytes from BALB/c mice immunized against leukemia EL4 of C57BL/6 mice were absorbed on polylysine-fixed EL4 monolayers. Nonadherent and adherent lymphocytes were collected and subjected to a second absorption. A third absorption was then performed on nonadherent and adherent cells recovered from the second absorption. While 69% of the cells recovered from the first absorption were nonadherent, two additional absorptions of nonadherent cells produced a population that was 94% adherent. Similarly, the proportion of adherent cells was increased from 31 to 51% by the three sequential absorptions. Similar experiments designed to remove graft-*versus*-host reactivity from normal lymphoid populations were unsuccessful. Newborn (C3H x C57BL)F_1 mice were inoculated with normal C57BL spleen cells incubated on fibroblast monolayers on the strains (C3H x C57BL)F_1, C3H, and C57BL/6. Other newborn mice were injected with syngeneic spleen cells or with C57BL/6 spleen cells incubated in empty petri dishes. At nine days postinoculation, spleen indices were similar in all groups. Exposure of normal lymphocytes to genetically appropriate immunoabsorbents also failed to prevent lethal secondary disease following x-irradiation. Specific fractionation of immunocompetent effector cells could prove valuable in transplantation and tumor immunology.

6268 IMMUNOLOGICAL REACTIVITY OF HUMAN RECIPIENTS OF SYNGENEIC AND ALLOGENEIC MARROW GRAFTS.

(E.) Thomas, E. D. (Univ. Washington, Sch. Med., Seattle), L. Fass, H. D. Ochs, E. M. Mickelson, R. Storb and A. Fefer. *Recent Results Cancer Res* 47:12-16, 1974.

The immunological reactivity of seven patients with bone marrow grafts was studied by measuring immunoglobulins, testing in mixed leukocyte culture, phage immunization and dinitrochlorobenzene (DNCB) skin testing. Three of the graft recipients had aplastic anemia and the other four had hematological malignancy but none showed significant graft-*versus*-host disease or recurrence of disease. Immunoglobulin levels appeared within normal limits, as did peripheral blood lymphocyte levels. One recipient of syngeneic marrow showed good production of circulating antibody against bacteriophage θX174 antigen with the normal pattern of IgM after primary immunization and IgG after secondary immunization. The other recipient of syngeneic marrow showed production of IgM after secondary immunization. The recipients of allogeneic grafts did not produce antibody to phage antigen. Skin tests to *Candida* and mumps, were negative in all patients before grafting, and were positive to *Candida* in one patient and his donor 2 yr after grafting. Reactivity to DNCB was not observed following allogeneic grafting. One HL-A-matched donor was sensitized to DNCB before grafting, but the recipient showed no reactivity after grafting. One identical twin was negative to DNCB 39 days after grafting, while another responded to DNCB two yr after grafting. In most cases, the *in vitro* response to donor lymphocytes in the recipients was normal to allogeneic cells and to phytohemagglutinin after methotrexate was discontinued. The results suggest a much slower recovery of immunologic function in the recipients of allogeneic grafts than in the recipients of syngeneic grafts.

6269 IMMUNOTHERAPY OF TRANSPLANTABLE, DIETHYLNITROSAMINE-INDUCED HEPATOMAS IN SYNGENEIC GUINEA PIGS.

(E.) Rapp, H. J. (Natl. Cancer Inst., Bethesda, Md.). *Biochem Dis* 4 (Part B):701-709, 1974.

Tumor suppression by *Mycobacterium bovis* (strain BCG) was demonstrated in strain 2 guinea pigs bearing transplantable hepatomas originally induced by diethylnitrosamine in syngeneic animals. BCG therapy was initiated following the observation that tumors did not develop at the site of i.m. injection in animals immunized (days 12 and 19) intradermally with doses of living tumor cells that produced delayed skin reactions; and after experiments demonstrating that immunologically-nonspecific components of the immune system had an inhibitory effect on tumor growth. On day seven after intradermal challenge with 10^6 living tumor cells, guinea pigs were treated by either intralesional injection of BCG, intralesional injection of diluent, or by tumor excision. Animals who received diluent and those whose skin tumors were excised died following widespread metastases. More than 1/2 the animals that received living BCG intralesionally were completely and permanently cured; those that eventually developed metastases lived significantly longer than tumor-bearing animals not treated with BCG. When treatment was withheld until day 12, only about 20% of the animals were cured. Histologic examination of draining lymph nodes several days after BCG injection showed the presence of a larger number of histiocytes and a few tumor cells that appeared to be degenerating. Draining lymph nodes of cured animals eventually returned to normal appearance. Acid-fast bacilli were also present in lymph nodes, indicating that BCG-lymphocyte interaction with migration inhibition factor release can take place in the lymph node as well as in the tumor transplant. In BCG-induced tumor regression, histiocytes may serve at least two purposes: the immunologically nonspecific destruction of tumor cells, and the mediation of a specific immune response leading to the appearance of lymphocytes specifically sensitized to tumor antigens. The possible application of these findings for the immunotherapy of human cancer are discussed.

6270 KINETICS OF SPECIFIC CELL-MEDIATED IMMUNE RESPONSE IN CANCER-BEARING HOSTS.

(E.) Barski, G. (Inst. Gustave-Roussy, Villejuif, France), J. K. Youn and D. Le Francois. *Recent Results Cancer Res* 47:54-57, 1974.

The mechanism of cell-mediated immunity in mice bearing syngeneic transplantable tumors is reviewed. In three different murine host-tumor systems, cell-mediated immunity (expressed as specific cytotoxicity of peritoneal cells (PC) directed against the target tumor cells *in vitro*) appeared at the beginning of palpable tumor growth, then declined and finally disappeared in mice bearing tumors exceeding an average diameter of 10 mm. Additional evidence indicated that this "eclipse" phenomenon in tumor-bearing animals was immunologically specific for different tumors. The mechanism responsible for the immunological "eclipse" phenomenon appears to be related to the presence of a "blocking factor" in the serum of immunologically "eclipsed" tumor-bearing mice. This factor was able to cancel out (to a large extent) the specific cytotoxicity of immunologically active PC from immunized mice. Thus, progressive tumor growth appears to be associated with the presence in the host's serum of a factor which blocks the specific "killer" activity of the sensitized lymphoid cells collected in ther peritoneal cavity. The block may operate as a central mechanism directed against the effector lymphoid cells, although the nature of the "blocking factor" is unclear at this time.

6271 ALTERATIONS IN IMMUNE RESPONSE TO EXPERIMENTAL MURINE TUMOR-ASSOCIATED ANTIGENS FOLLOWING ADMINISTRATION OF BCG OR POLYSACCHARIDE FROM *PROTEUS VULGARIS*.

(E.) Donner, M. (Res. Unit Exp. Cancerol. Radiobiol., INSERM, Vandoeuvre-les-Nancy, France) and D. Vaillier. *Recent Results Cancer Res* 47:308-319, 1974.

The effects of Calmette Guérin bacillus (BCG) and a polysaccharide extracted from *Proteus vulgaris* on the growth of chemically-induced rhabdomyosarcomas in inbred C_3H/He mice previously sensitized to tumor-associated antigens were studied. Two weeks after the second of two tumor immunizations, the mice were injected (i.p.) twice with either BCG (0.6 mg) or polysaccharide from *P. vulgaris* (0.1 mg). The *in vivo* level of antitumor immunity, enhancing properties of the serum, and release of lymphotoxins by the spleen cells were determined at intervals of 1-46 days after the last injection of adjuvant. BCG and the polysaccharide from *P. vulgaris* induced similar modifications in the immune response to the murine solid-tumor-associated antigens. The effect of both adjuvants on the level of *in vivo* antitumor immunity involved: strong depression of antitumor immunity in the early days after adjuvant treatment, subsequent return to a normal level of immunity and a later significant increase in the immune response of the animal to tumor-associated antigens. The decline in antitumor immunity was associated with a marked change in the properties of the animals' serum. In the early days following adjuvant administration, humoral substances were able to stimulate the growth of solid tumors. The enhancing capacity of the serum was greatly reduced when sera were collected 22 days after adjuvant treatment. The adjuvant treatments influenced the release of lymphotoxins from spleen cells sensitized to tumor antigens: in the early days after adjuvant administration the production of nonspecific cytotoxic factors was very weak; 2 weeks later the cell-mediated immune response had returned to a normal level and after one month the immune response had increased above control levels. At present, these results cannot be directly extrapolated to other systems.

6272 ANALYSIS OF TUMOR-SPECIFIC CELL-MEDIATED IMMUNE REACTIONS IN THE PRIMARY AUTOCHTHONOUS HOST. *(E.)* Kikuchi, K. (Sapporo Med. Coll., Japan), Y. Ishii, H. Ueno, T. Kanaya and Y. Kikuchi. *Gann Monogr Cancer Res* 16:99-116, 1974.

Quantitative and morphological techniques were used to study tumor-specific lymphoid cell-mediated immune reactions against autochthonous 3-methylcholanthrene (MCA)-induced sarcomas in the Wistar-King-Aptekman or Lewis strain rats. Cell-mediated tumor-specific immune resistance was clearly demonstrated by *in vivo* and *in vitro* in autochthonous tumor-host systems using MCA-induced sarcomas. Serum cytotoxicity was only rarely observed, even with serum from highly resistant hosts. Electron microscopic studies revealed that the effector cells in these reactions were lymphocytes and millipore membrane studies suggested the possible production of a diffusible cytotoxic substance(s) by immune lymphocytes. The *in vitro* cytotoxicity of the lymphocytes closely paralleled the degree of transplantation resistance to autochthonous tumor in the primary host. In human cancer patients, the destruction of tumor cells by autologous lymphocytes appeared to correlate well with the patient's resistance to his own cancer. The results suggest that cell-mediated responses are primarily involved in the rejection of tumor autografts in laboratory animals and imply that estimation of a patient's immunological state of resistance to cancer may be possible using *in vitro* laboratory tests.

273 THE ROLE OF SENSITIZED LYMPHOCYTES AND MACROPHAGES IN CELL-MEDIATED IMMUNITY GAINST TUMOR GRAFTS. *(E.)* Nomoto, K. (Sch. Med., yushu Univ., Japan), H. Yamada, S. Muraoka and K. akeya. *Gann Monogr Cancer Res* 16:89-98, 1974.

he roles of sensitized lymphocytes and macrophages n *in vivo* cell-mediated reactions against tumor llografts were studied. An allotransplantable ymphoma which originated in an outbred golden hamter was used as a source of antigenic target cells. large number of macrophages and a small number f lymphocytes were detected around the lymphoma rafts in adult MHA hamsters. The primary cytooxic effector cells proved to be lymphocytes rather han macrophages. Tubercle bacilli-sensitized hamter lymphocytes exerted a cytotoxic effect on puriied protein derivative (PPD)-coated hamster lymhoma cells. The cytotoxic effect appeared to deend on nonspecific cytotoxic factor(s) released by he sensitized lymphocytes after specific binding ith the corresponding antigens. MHA hamsters deeloped cytotoxic activity and delayed hypersensiivity against the lymphoma cells after allograftng; the accumulation of macrophages around these rafts appeared to be an expression of delayed hyersensitivity against the allogeneic cellular antien. Delayed hypersensitivity against lymphoma ells was also developed in golden hamsters immunized ith lymphoma antigen in Freund's complete adjuvant, ut not in those grafted with live lymphoma cells. n the other hand, cytotoxic activity was detected nly in the hamsters receiving live lymphoma cell grafts. The results suggest that the two typical types of cell-mediated immunity, cytotoxic activity and delayed hypersensitivity, might depend on different subpopulations of sensitized lymphocytes.

6274 CELLULAR IMMUNITY AGAINST ROUS SARCOMAS OF CHICKENS. PREFERENTIAL REACTIVITY AGAINST AUTOCHTHONOUS TARGET CELLS AS DETERMINED BY LYMPHOCYTE ADHERENCE AND CYTOTOXICITY TESTS *IN VITRO*. *(E.)* Wainberg, M. A. (Hebrew Univ. Med. Sch.-Hadassah Hosp., Jerusalem, Israel), Y. Markson, D. W. Weiss and F. Doljanski. *Proc Natl Acad Sci USA* 71(9):3565-3569, 1974.

In order to compare splenocyte-target cell interaction in both autochthonous and allogeneic confrontations between Rous sarcoma (RS) cells and effector cells, six-to-ten-week-old white Leghorn chickens were injected with 10^6 focus-forming U of Rous sarcoma virus. Three to four weeks later, spleens were removed; normal chickens served as control spleen donors. Interaction was measured using a new quantitative immunoadherence test in which splenocytes (10^8 cells) were labeled by incubation in insulin medium containing 70 µCi of ^{51}Cr for 30 min at 37 C. Radioactivity was determined by spectrophotometry, aliquots were added to target cells at a splenocyte/RS ratio of 50:1 or 100:1 and the cultures were incubated at 37 C for varying time periods. Microcytotoxicity assays, in which ^{51}Cr-labeled RS cells were incubated with suspensions of splenocytes at 37 C and the amount of label released was measured, were used to determine the specificity of RS target cell killing by splenocytes from tumor-bearing donors. There was no significant difference in the amounts of ^{51}Cr released from monolayers of normal chicken embryo cells interacting with normal or sensitized splenocytes. RS cells showed greater reactivity with autocthonous tumor cells than with allogeneic cells in the adherence test. After one hr incubation, a clustering of splenocytes on the target monolayer was consistently evident in the autochtonous combination, and less evident in the allogenic one. The same results occurred when both tests were performed simultaneously on identical cell populations. Splenocytes showing greatest adherence were also most cytotoxic, indicating that adherence may be concomitant to, and possibly a forerunner of, target cell injury. These findings indicate that there is an individual-specific aspect of host reactivity to Rous sarcoma cells.

6275 EVIDENCE FOR *IN VIVO* PROTECTION AGAINST MURINE-SARCOMA VIRUS-INDUCED TUMORS BY T LYMPHOCYTES FROM IMMUNE ANIMALS. *(E.)* Gorczynski, R. M. (Imp. Cancer Res. Fund, London Univ. Coll., England). *J Immunol* 112(2):533-539, 1974.

The T cell role in the *in vivo* response to murine sarcoma virus (MSV)-induced tumors was studied using male BALB/c mice. Tumors were induced by a thigh injection of 0.1 ml of tumor homogenate containing MSV and murine leukemia virus complex of Moloney. Testing utililized both rabbit antimouse brain-associated antigen (Br anti-θ) and rabbit antimouse immunoglobulin (anti-Ig) antisera. All viable cell determinations were made using trypan blue exclusion. The cytotoxicity of the antisera was tested in the presence of guinea pig complement for BALB/c thymus and BALB/c lymph node cells. Br anti-θ killed 100% of BALB/c thymocytes and 70% of BALB/c lymph node cells, while anti-Ig killed only negligible thymocytes and 35% of lymph node cells. The suppression of enhanced early protein synthesis in mouse spleen cells in leucine-free, serum-free minimal essential medium with either PHA (T cell mitogen) or LPS (B cell mitogen) showed that the Br anti-θ abolished PHA response but did not affect LPS response, while anti-Ig had the reverse effect. Antibody response to sheep erythrocytes (SRBC) and to dinitrophenyl-conjugated polymerized flagellin (DNP-POL) was tested using treated (either with Br anti-θ serum or anti-Ig serum) spleen cells of BALB/c mice. Br anti-θ abolished SRBC response but had no effect on anti-DNP-POL response, while anti-Ig abolished both responses. SRBC response was reconstructed from Br anti-θ treated spleen cells by addition of SRBC-educated thymus cells; this was not possible in anti-Ig treated spleen cells. Bone marrow cell addition reconstructed anti-SRBC and anti-DNP-POL response in anti-Ig treated spleen cells but had no effect in Br anti-θ treated cells. The production of lethal tumors in irradiated (700 r) mice receiving MSV was noted. The injection of large numbers of immune spleen cells (order of 10^8) with MSV caused complete ablatement of tumor development. Normal spleen cell injections with MSV did not provide protection, yet 5 x 10^6 immunized cells protected the animals from tumors. The protection was abolished by treatment of immune cells with Br anti-θ serum and complement. Treatment with immune cell anti-Ig serum caused no decrease in protection, but enhanced tumor regression. The results suggest that T lymphocytes are the main mediators of protection in MSV-induced sarcoma.

6276 DISTURBANCE OF IMMUNE SURVEILLANCE IN TUMOR-BEARING HOST.

(E.) Kitagawa, M. (Osaka Univ. Med. Sch., Japan), T. Hamaoka, K. Takatsu, S. Haba, U. Yamashita and H. Masaki. *Gann Monogr Cancer Res* 16:45-52, 1974.

The cellular mechanism of immunosuppression in tumor-bearing animals was analyzed in terms of the thymus-dependent (T) and bone marrow-derived (B) lymphoid cells using hapten-protein carrier conjugates as antigens. In mice bearing Ehrlich ascites tumors, the immunological activity of the T cells was estimated quantitatively by the helper activity for the anti-hapten antibody response by hapten-primed lymphoid cells. In this system, the development of carrier-primed helper T cells was markedly suppressed in mice bearing the Ehrlich ascites tumor. The nuclear fraction, chromatin fraction and cell-free ascites fluid of the Ehrlich carcinoma suppressed the development of helper T cells in mice when tested using this system. The experimental results suggested that nuclear components such as histone fraction were released from the tumor cells and acted as immunosuppressive agents on lymphoid organs in the tumor-bearing host. The suppressed T cell activity in tumor-bearing mice was restored by the administration of the antitumor polysaccharide, lentinan. The results further suggested that the enhancement of T cell activity by lentinan may have been instrumental in the suppression of tumor growth in the treated animals.

6277 IMMUNOLOGICAL STUDIES IN ACUTE LEUKEMIA.

(E.) Santos, G. W. (Dep. Med., Johns Hopkins Univ., Baltimore, Md.), G. M. Mullins, W. B. Bias, P. N. Anderson, K. D. Graziano, D. L. Klein and P. J. Burke. *Recent Results Cancer Res* 47:17-24, 1974.

The immunological responsiveness or untreated acute leukemia patients and their families to acute leukemia cells was studied. The subjects included 12 patients with acute myelogenous leukemia (AML), 13 patients with acute myelomonocytic leukemia (AMMOL), one patient with acute progranulocytic leukemia (APGL), four patients with acute lymphocytic leukemia (ALL), 17 parents of leukemia patients, 75 siblings and 20 unrelated controls. The immunological studies involved skin testing, HL-A typing and testing for cytotoxic antibody, one-way mixed leucocyte culture (MLC) testing, ^{51}chromium release cytotoxicity testing and macrophage migration inhibition testing. The acute leukemia patients were frequently unresponsive to the antigens tested in the skin tests but in no instance was a delayed hypersensitivity response to intradermally injected autologous tumor cells found. Nine normal siblings were found to be HL-A identical to their leukemic siblings and eight of these responded to leukemic blasts in the MLC tests. In addition, positive cytotoxicity indicated by ^{51}Cr release from leukemic blasts was seen in six of 43 family members tested and inhibition of macrophage migration by supernatants obtained from family members' lymphocytes cultured with leukemic blasts was seen in six of 34 MIF tests. There was no evidence for "blocking" activity in the patient sera. Finally, the sera of one father and two brothers of leukemic patients were cytotoxic to acute lymphoblastic leukemic cells. The results strongly suggest a viral etiology for acute leukemia.

6278 BACILLUS CALMETTE-GUERIN AND ANTILYMPHOCYTE SERUM IN CARCINOGENESIS: EFFECTS ON THE HAMSTER POUCH.

(E.) Giunta, J. L. (Tufts Univ. Sch. Dent. Med., Boston, Mass.), A. E. Reif and G. Shklar. *Arch Pathol* 98(4):237-240, 1974.

Immunosuppression with rabbit antilymphocyte serum (ALS) should enhance and immune stimulation with bacillus Calmette-Guérin (BCG) should delay carcinogenesis. Using the dimethyl benzanthracene (DMBA)-buccal pouch model system for chemical carcinogenesis, 94 hamsters were divided into groups receiving DMBA alone or DMBA plus normal rabbit serum (0.5 ml, i.p. 21 inj. over a 7-wk-period), ALS (0.5 ml, i.p., 21 inj. over a 7-wk-period), or BCG at two dosage levels (1 mg in 0.5 ml, s.c., for 14 inj. over a 7-wk-period and 1 mg in 0.5 ml, s.c. for 7 inj. over a 70 day period). After killing half of the animals at eight and 12 weeks, each pouch was examined histologically and was assigned am empirical score representative of the most advanced pathologic change. Statistical analyses were performed. At 8 weeks, ALS greatly enhanced carcinogenesis, whereas the BCG (higher dosage) retarded carcinogenesis. At 12 weeks, BCG (both dosages) retarded carcinogenesis. These results indicate that ALS enhances DMBA carcinogenesis whereas a sufficient dose of BCG delays it.

6279 IMMUNOGLOBULIN SYNTHESIS IN TUMORS OF HUMAN LYMPHOID ORIGIN SERIALLY TRANSPLANTABLE IN THE NEONATAL SYRIAN HAMSTER. *(E.)*

Pothier, L. (Harvard Med. Sch., Boston, Mass.), P. Parke and R. A. Adams. *Int J Cancer* 14(2):198-206, 1974.

To relate immunoglobulin production to certain aspects of the neoplastic behavior of human lymphoid cells proliferating *in vivo*, the immunoglobulin cell products from 21 human tumor lines experimentally maintained in neonatal hamsters were analyzed. These lines were established in normal and ALS-treated Syrian hamsters by direct implantation of cells from the patient or after an intermediate stage in cell culture. Three lines derived from patients with acute lymphoblastic leukemia (conversion from lymphosarcoma) which also produced leukemic manifestations in the hamster, did not produce immunoglobulins. However, with the other lines, derived from patients with lymphosarcoma (without conversion to leukemia), infectious mononucleosis, Hodgkin's disease, and Letterer-Siwe's disease, human immunoglobulin components were detected in the hamster sera by immunodiffusion techniques. With rare exceptions, H-chain was accompanied by L-chain; these components being pecific for the tumor cell line under investigation nd without variation as to the class of H-chain or he type of L-chain detected throughout the observaion period (three months to two yr). One line, erived from a patient with infectious mononucleosis, howed a sequential deletion of both H- and L-chains; nd in another line (also derived from patient with nfectious mononucleosis), an H-chain not usually presnt was detected for a short time midway in serial pasage. The results suggest that the environment may ncourage a multipotential cell to differentiate long certain lines or select a certain cell type rom the heterogeneous population implanted into the amster. They also suggest that the underlying disase and stage of the disease may influence the synhesis of immunoglobulin.

280 IgM-PRODUCING TUMORS IN THE BALB/c MOUSE: A MODEL FOR B CELL MATURATION. *(E.)*

ndersson, J. (Basel Inst. Immunol., Switzerland), . Buxbaum, R. Citronbaum, S. Douglas, L. Forni, . Melchers, B. Pernis and D. Stott. *J Exp Med* 0(3):742-763, 1974.

ansplantable murine tumors (McPc 1748, TEPC 183, Pc 774, Y 5781, and W 3469) were grown s.c. in LB/c mice. The state of maturation of cells tained from the subsequent IgM-producing lymphotomas was studied using a variety of morphologil and biochemical criteria, i.e., electron microopy, analysis of the distribution of surface-und and intracytoplasmic IgM using immunofluoresnce, and the biochemical study of IgM synthesis, turnover, and secretion. The degree of maturity was assessed by comparison with normal mitogen-stimulated B lymphocytes. The cells of different tumors appeared to represent different stages in B-cell maturation. Thus, McPc 1748 cells resembled 10-25-hr normal, lipopolysaccharide-stimulated B cells; W 3469 cells resembled 20-35-hr stimulated B cells; TEPC 183 cells resembled 45-65-hr stimulated B cells; Y 5781 cells resembled 80-110-hr stimulated B cells; and McPc 744 cells resembled 100-130-hr stimulated B cells. The tumors were categorized as group I (McPc 1748 and W 3469) and group II (TEPC 183, Y 5781 and McPc 774). Group I cells had strong surface immunofluorescence with anti-μ antiserum, few cytoplasmic membrane-bound polysomes, actinomycin D-sensitive synthesis of only a small portion of total cell protein as IgM. IgM turnover was slow, intracellular equilibration of labeled precursors was difficult to attain, only a very small amount of 19S IgM was secreted and intracisternal A particles were not detectable with electron microscopy. Group II cells had little or no membrane immunofluorescence, abundant intracytoplasmic Ig (similar to that of plasmablasts or young plasma cells); a large portion of the total cell protein was IgM; IgM turnover was rapid, secretion copious and apparently its synthesis was relatively actinomycin D-resistant; intracisternal A particles were numerous. Ability to synthesize Ig is not absolutely required for the presence of intracisternal A particles, and in this respect, cells of groups I and II may differ quantitatively rather than qualitatively. Thus, there may be some process involved in B-cell maturation which allows the flagrant expression of these presumably viral elements only by the more mature members of the series. The IgM-producing BALB/c tumors should serve as a useful model for examining the process of B-cell maturation which results in the IgM antibody-secreting cell.

6281 IMMUNOGLOBULIN ANALYSIS IN FAMILIES OF MYELOMA PATIENTS. *(E.)*

Odeberg, H. (Dep. Intern. Med., Univ. Lund, Sweden), B. G. Johansson and S. O. Berlin. *Acta Med Scand* 196(5):361-367, 1974.

To determine whether an improperly regulated and/or hyperreactive plasma cell system might be operating within familial aggregations of myeloma cases, the electrophoretic plasma protein and immunoglobulin patterns were studied in 24 members of two families with known familial occurrence of myeloma and in 124 relatives of ten patients with non-familial myeloma. The immunoglobulin levels were also determined in 54 normal blood donors. Venous serum samples were studied by immunoelectrophoresis and radial immunodiffusion, and in some cases urine samples were also studied by electrophoresis. In one of the families with multiple occurrence of myeloma, the disease was found in a mother and son. In the other family, two siblings had myeloma and one sibling had an asymptomatic M-component. No further M-component was found among the 24 members of these families. In two of the other ten families, an M-component was found in an asymptomatic relative. Similarly, the distribution of immunoglobulin levels among the various members of the 12 families differed little from that found among the controls, and very few markedly deviant immunoglobulin levels were found. Thus, there was no evidence of an elevated incidence of M-components or of a defective regulation of immunoglobulin production within the myeloma families studied. Furthermore, there is no support for the suggested existence of an improperly regulated and/or hyperactive plasma cell system among such families.

6282 ISOLATION OF DESIALYLATED AND LOW IODINATED THYROGLOBULIN IN AN EXPERIMENTAL RAT THYROID TUMOUR.

(E.) Monaco, F. (2nd Med. Clin., Univ. Rome, Italy), S. Grimaldi, G. Scuncio, and M. Andreoli. *Acta Endocrinol (Kbh)* 77(3):517-526, 1974.

The isolation, chemical and other characteristics of a thyroglobulin from rat thyroid tumor tissue was described and compared with normal thyroid thyroglobulin. The most striking difference was the 10-fold decrease in iodine and sialic acid content of tumor thyroglobulin. Thyroglobulin was isolated by ammonium sulfate fractionation of a supernatant fraction of thyroid tissue homogenate, followed by fractionation via sucrose linear gradient centrifugation. The 18S fraction corresponds to tumor thyroglobulin and was chosen for further study by gel electrophoresis, immunoelectrophoresis, immunodiffusion and sialic acid analysis. Tumor thyroglobulin gave results in these tests very similar to those for normal thyroglobulin that had been desialylated. This tumor thyroglobulin lacks thyroxine but does contain mono- and diodo-tyrosines; its thyroglobulin secretory activity is about 10^{-2} that of normal thyroglobulin. The low sialic acid and iodine content is considered related to the loss of hormone function of this thyroglobulin.

6283 GM POLYMORPHISM AND LEUKEMIA.

(E.) Nevo, S. (Dep. Biol. Haifa Univ., Israel), I. Tatarsky and H. H. Fudenberg. *Isr J Med Sci* 10(12):1530-1535, 1974.

One hundred and fourteen patients with leukemia (94 cases of chronic lymphocytic leukemia and 20 cases of acute leukemia) were typed for IaG-1 markers Gm(a,x,z and f), IgG-3 markers Gm(b^0, b^1, b^3, c^3, c^5, g, s, and t) and Inv-1 markers in order to determine the relationship of these antigenic markers to leukemia. Leukemia patients were not different from controls (matched for sex and national origin) with respect to the markers studied, but the relationship between leukemia and Gm type is not conclusive due to the relatively small sample of patients used. The differential fitness of different genotypes within the single polymorphism studied may be of the order of magnitude which requires very large samples for its detection.

6284 AUTOANTIBODIES IN THE SERA OF VARIOUS CANCER PATIENTS.

(E.) Yoshida, T. O. (Aichi Cancer Cent. Res. Inst., Nagoya, Japan). *Gann Monogr Cancer Res* 16:63-70, 1974.

The presence of autoantibodies to normal tissue in the sera of various cancer patients was evidenced by the following factors. First, considered in relation to the clinical course and treatments, is the high incidence of heterophilic antinuclear antibodies (ANA) in patients with nasopharyngeal cancer and other tumor types which have been found within nuclei of human and animal cultured cells. A statistical analysis of ANA-positive cases showed that the proportion of cases with NPC was by far the highest; the cases with malignant neoplasma of hematopoietic organs were very low; and the cases with other cancer were at a level between those of NPC and hematopoetic neoplasma. Second is the correspondence of antigens to ANA (autochthonous and homologous cultured cells) in the cell nuclei of mice, rabbits, rats and monkeys. Third is the fluorescence patterns showing localized ANA-corresponding antigens. Fourth are the immunoglobulin classes, IgG and IgM alone and in combination, found in NPC sera and ANA titers which are relatively low (1:10 and 1:160). Fifth is the incidence of ANA after inoculation with Graffi and Rauscher leukemogenic virus, correlated with the infectivity of the viral inoculum and its leukemogenesis. Sixth are the anticytoplasmic antibodies also found in sera of patients with tumors, some of which react with nerve fibers and fascicles in muscle tissue. Other autoantibodies to various tissue components are also present. Seventh is the cross reactivity of ANA with cancer and autoimmune diseases as shown by blocking tests and ANA absorption with purified substances.

6285 SPECIFICITY OF ANTIBODIES TO NONHISTONE CHROMOSOMAL PROTEINS OF CULTURED FIBROBLASTS.

(E.) Zardi, L. (Temple Univ. Sch. Med., Philadelphia), J. C. Lin, R. O. Petersen and R. Baserga. *Cold Spring Harbor Conf Cell Proliferation* 1:729-741, 1974.

The value of antibodies to nonhistone chromosomal proteins in examining diversity has been shown; this investigation utilized antibodies to chromatin of WI-38 human dipolid fibroblasts and antibodies to chromatin of 3T6 mouse fibroblasts to analyze: (1) the possibility of obtaining antibodies to chromosomal proteins from different species of cultured fibroblasts, (2) species specificity of antisera to chromatin, (3) antibodies elicited against chromatin components and (4) isolation of antibodies specific for a given chromosomal fraction. Chromatin was prepared from either 3T6 mouse fibroblasts or WI-38 human fibroblasts by standard methods. New Zealand white rabbits and chickens were immunized by four intradermal injections of 400 µg DNA as chromatin. One week after the last injection in rabbits (four days after in chickens) a s.c. booster injection was given and antisera were harvested seven days following this injection. Complement fixing antibodies in the immune sera were obtained from 3T6 chromatin and WI-38 chromatin-immunized rabbits and WI-38 chromatin-immunized chickens (3T6 chromatin immunization of chickens was not done). Rabbit antibodies to 3T6 chromatin were shown to distinguish between antigens of 3T6 chromatin and those of WI-38 chromatin. Similar results were obtained from chicken antiserum. Rabbit antiserum to WI-38 reacted at its highest dilution with WI-38 chromatin and at lower dilutions with nonhistone chromosomal proteins from WI-38, histone from WI-38, 3T6 chromatin, nonhistone proteins of 3T6, histones from 3T6 cells and DNA, resp., using a 50% complement fixation as a reference. Antibodies which reacted to antigens common to WI-38 and 3T6 chromatin were shown to be separable using absorption of antibodies to antigens coupled to a CNBr-sepharose 4B column, and using chicken precipitins for antibody precipitation. The results confirm previous work indicating that nonhistone chromosomal proteins of different tissues in the same species can be distinguished by biochemical characteristics. The modest immunogenicity of histones was confirmed. Complement fixing antibodies to nonhistone chromosomal proteins were shown to be species specific. The use of antibodies to chromatin in the immunological identification of nonhistone chromosomal proteins specific for similar tissues from different species is clearly indicated.

6286 TUMOUR-SPECIFIC ANTIBODY NEUTRALIZATION OF FACTORS IN RAT HEPATOMA-BEARER SERUM WHICH ABROGATE LYMPH-NODE-CELL CYTOTOXICITY.

(E.) Robins, R. A. (Cancer Res. Campaign Lab., Nottingham England) and R. W. Baldwin. *Int J Cancer* 14(5): 589-597, 1974.

The abrogation of the cytotoxicity of immune lymph-node cells when added to target cells (blocking) or effector cells (inhibition) was examined in the rat hepatoma system. The hepatomas used were induced by 4-dimethylaminobenzene in Wistar rats and were maintained by serial s.c. passage in syngeneic recipients. Sensitized lymph-node cells were obtained from syngeneic immunized rats which had previously rejected two monthly tumor challenges of 2×10^5 viable tumor cells. Tumor-bearer (TB) sera were obtained from rats with 14-28 day old hepatoma transplants. Post-excision (PE) sera were taken from rats 7-10 days after surgical resection of an s.c. hepatoma transplant. Hepatoma-immune sera were obtained from rats used as donors of sensitized lymph-node cells. Rabbit antiserum (RS) to hepatoma D23 was raised by immunization with cells from an ascitic variant of hepatoma D23. RS was absorbed *in vivo* before use *in vitro*. Serum blocking and serum inhibition of lymph-node cell cytotoxicity were measured. The blocking activity of the TB sera was reduced or abolished when TB and PE sera were mixed in equal proportions. This was a relative effect as blocking reappeared at a 1/10 (resp.) dilution. Mixtures of hepatoma immune and TB sera were obtained which did not block immune lymph-node cell cytotoxicity. Similar results occurred using RS directly. The effect with hepatoma immune sera was more pronounced than the PE effect. PE sera neutralized TB sera inhibition of lymph-node cell cytotoxicity, with the effect being tumor-specific (D30 TB sera could only be neutralized by D30 PE sera). The results show that the abrogation of hepatoma immune lymph-node cell cytotoxicity by hepatoma-bearer sera can be counteracted by three types of sera, all known to contain antibody to tumor antigen. Counteraction of both blocking and inhibition of lymph-node cell cytotoxicity is reported.

6287 SIMIAN SARCOMA AND FELINE LEUKEMIA VIRUS ANTIGENS: ISOLATION OF SPECIES- AND INTERSPECIES-SPECIFIC PROTEINS.

(E.) Hoekstra, J. (Dep. Microbiol., Rush-Presbyterian-St. Luke's Med. Cent., Chicago, Ill.) and F. Deinhardt. *Intervirology* 2: 222-230, 1973/74.

Total viral proteins were prepared by Tween 80-ether disruption of purified virus from feline leukemia virus (FeLV)-infected cat thymus cells and simian sarcoma virus type 1/simian sarcoma-associated virus type 1 (SSV-1/SSAV/1)-infected marmoset skin cells. The molecular weights of the major proteins of FeLV and SSV-1/SSAV-1 were determined on SDS polyacrylamide gels. Chromatography of FeLV in 6 M GuHCl yielded 2 glucosamine-labeled glycoproteins (gp-100, gp-70) and four other proteins (p27, p14, p12, p10) lacking glucosamine; SSV-1/SSAV-1 yielded three glycoproteins (gp-100, gp-70, gp-60) and three other proteins (p30, p14, p12). In immunodiffusion tests, FeLV p27 and Fe LV p14 each formed one precipitation line against a goat serum containing both species-specific and interspecies-specific antibodies; p14 lacked any demonstrable antigenic cross-reactivity with FeLV p27. Similarly, SSV-1/SSAV-1 p30 was found to contain an interspecies and species specific antigen; p30 was the major protein of the virus, analogous to FeLV p27. The SSV-1/SSAV-1 p30 also formed a line of identity with another primate C-type virus gs antigen preparation (gibbon ape lymphoma) in immunodiffusion tests against a goat antiserum to SSV-1/SSAV-1, but reacted only weakly with antisera against gs antigens of murine and feline C-type viruses. Two additional proteins of SSV-1/SSAV-1 also reacted with lines of identity with the corresponding antigens of GAL virus but not at all with other mammalian viruses. The results indicate that the use of purified non-human primate C-type virus antigens and their corresponding antibodies may lead to the identification of antigens or antibodies of human C-type viruses.

6288 IMMUNOFLUORESCENCE ANTIBODIES IN HUMAN SERA TO ANTIGENS IN CELLS OF A TYPE C VIRUS PRODUCING HUMAN CULTURE OF TUMOR ORIGIN.

(E.) Priori, E. S. (Univ. Texas Syst. Cancer Cent., Houston) and L. Dmochowski. *Prog Exp Tumor Res* 19:182-193, 1974.

Sera of 345 patients with a variety of malignant diseases were studied for reactivity with cells of a line (ESP-1) derived from pleural effusion cells of a patient with Burkitt's lymphoma (American type). Spontaneous production of type C virus by ESP-1 cell cultures had been observed for more than 100 subpassages. Results of immunofluorescence tests showed that sera of about 42% of the patients reacted with ESP-1 cells compared with 1% of sera from normal donors (180). The reaction consisted of fluorescence in the cytoplasm of fixed cells, located either perinuclearly or in the entire cytoplasm. Reactivity was highest with sera of patients in the following tumor categories: Burkitt's lymphoma (90%), reticulum cell sarcoma (90%), rhabdomyosarcoma (74%), carcinoma of the bladder (63%), giant cell tumors (66%), and chrondrosarcoma (66%). Sera of 50% of patients with Hodgkin's disease and chronic lymphocytic leukemia showed similar reactions. Reactivity was lower (20-43%) with sera from patients with bone tumors, liposarcoma, breast carcinoma, nasopharyngeal carcinoma, acute leukemia, and lymphoma. The positive reaction of sera could be removed or greatly diminished by absorption with cell packs of ESP-1 cells and cell packs of human cultures originating from osteosarcoma and giant cell tumor of the bone, by absorption with concentrated ESP-1 virus preparations, and by absorption with feline leukemia virus preparations. The indication that the positive reactions were due to tumor and/or viral antigens, including some group-specific-3 protein, strengthens the hypothesis that viruses are involved in the origin of human tumors.

6289 INHIBITION OF STEROID INDUCIBLE TYROSINE AMINOTRANSFERASE BY MOUSE AND RAT INTERFERON IN HEPATOMA TISSUE CULTURE CELLS. *(E.)* Beck, G. (Inst. Physiol. Biol. Chem., Strasbourg, France), P. Poindron, D. Illinger, J. P. Beck, J. P. Ebel and R. Falcoff. *FEBS Lett* 48(2):297-300, 1974.

The effect of interferon was studied on the induction of tyrosine aminotransferase in cultured rat hepatoma cells by response to steroids. Addition of interferon derived from rat fibroblasts, mouse L cells or rat serum to cell cultures 15 hr prior to stimulation by dexamethasone (10^{-7} M) produced a 14-34% inhibition in the rapid induction of tyrosine aminotransferase within the first 10 hr and also resulted in a 33-54% decrease in the maximum level of enzyme induction attained over the next 13 hr. Cell division and cellular protein synthesis were concomitantly inhibited by interferon treatment. These results support an inhibitory effect on translation of cellular m-RNA.

6290 INHIBITION OF MURINE-LEUKEMIA VIRUS PRODUCTION IN CHRONICALLY INFECTED AKR CELLS: A NOVEL EFFECT OF INTERFERON. *(E.)* Friedman, R. M. (Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, Md.) and J. M. Ramseur. *Proc Natl Acad Sci USA* 71(9):3542-3544, 1974.

The effect of interferon on the replication of mouse leukemia viruses was studied using AKR cells that had spontaneously become producers of a murine leukemia virus. When interferon at concentrations of 1-80 ml international mouse reference units/ml was added to AKR cell cultures, inhibition of endogenous virus production and viral reverse transcriptase occurred which varied directly with the concentration. Antiviral activity decreased over a 74 hr period. Contrary to expectation, intracellular viral p30 group-specific antigens were increased 4- to 6-fold. These findings suggest that interferon inhibited virus assembly or release in these AKR cells and not virus-directed transcription or translation.

6291 DETECTION OF TROPHOBLAST SPECIFIC ANTIGEN IN NORMAL AND NEOPLASTIC TROPHOBLAST. *(E.)* Tomoda, Y. (Sch. Med., Nagoya Univ., Japan), N. Saiki, S. Goto, Y. Kawashima and N. Ishizuka. *Acta Obstet Gynaecol Jpn* 20(1):14-18, 1973.

In order to elucidate the specific function of the trophoblast, isolation of organ specific antigen in trophoblast was investigated by immunochemical methods. Normal trophoblast and trophoblastic neoplasias, which were histologically choriocarcinoma (eight cases), chorioadenoma (five cases) and hydatidiform mole (eight cases), were used. Antisera against the trophoblast was prepared by immunization of rabbits with microsomal fraction of normal trophoblast cells. Analysis of antigenicity by agar gel diffusions showed the original antiserum against term trophoblast to yield many precipitin lines with sodium deoxycholate (SDOC)-soluble fraction of normal trophoblasts. Liver and human serum precipitins were also noted, so absorption of antisera was carried out to remove the antibodies against other proteins. Following absorption, precipitin lines against control antigens disappeared leaving one line between the absorbed antiserum and the SDOC-soluble fraction. Hemagglutination inhibition testing revealed trophoblast specific antigen to be present in normal pregnant trophoblasts (and trophoblastic tumors) but not in any other organs tested. Fluorescent antibody analysis revealed a localized fluorescence in the syncytium cells of the trophoblast. Intense fluorescence was noted in the normal trophoblast at 2-3 months and in hydatidiform mole. Fluorescence decreased with the maturity of the normal trophoblast. Fluorescence intensity was variable in choriocarcinoma. The specific inhibition test of immunofluorescent staining following absorption of antisera with trophoblast was negative. The results suggest that a trophoblast specific antigen exists in normal and neoplastic trophoblasts.

6292 ANTIGENIC EXPRESSION AS A DETERMINING FACTOR OF TUMOR GROWTH IN GROSS' VIRUS LYMPHOMA. *(E.)* Ioachim, H. L. (Lenox Hill Hosp., New York, N. Y.), S. Keller, M. Sabbath and B. Dorsett. *Prog Exp Tumor Res* 19:284-286, 1974.

A serially transplantable lymphoma cell line (550) in W/Fu rats and a tissue-culture line (550 CA) were studied comparatively by syngeneic transplantation. The two cell lines originated from the same tissue-culture line and were morphologically indistinguishable, but replication of Gross lymphoma virus and viral antigen expression occurred only in the 550 CA line. Immature (2- to 6-day-old) W/Fu rats were injected i.p. with 550 CA cells (100,000) or the same amount of 550 cells. Adult (two to three-month-old) rats were injected s.c. with 550 CA or 550 cells (500,000 each); s.c. with 550 CA cells on one side and 550 cells on the other; i.p. with 550 CA cells (500,000) or the same amount of 550 cells; and i.v. with a similar amount (300,000) of 550 or 550 CA cells. In infant rats, both 550 and 550 CA cells grew progressively and killed their hosts. In mature rats 550 cells, both s.c. and i.p. injected, grew progressively while 550 CA cells were consistently rejected. In the same host, large tumors formed on the side of inoculation of 550 cells and no tumors on the side of inoculation of 550 CA cells. Rats injected i.v. with 550 cells had numerous blood-borne metastatic tumors in lungs, kidneys, and adrenals. The capacity of the two cell lines for syngeneic transplantation correlated well with their expression of murine leukemia virus (MuLV) antigens. The 550 cells (consistently accepted upon grafting) showed no virus particles, were negative for MuLV antigens and were unable to induce the formation of anti-MuLV antibodies. In contrast, 550 CA cells (consistently rejected by adult rats) contained mature and immature virus particles, expressed MuLV antigens, and elicited the formation of anti-MuLV sera containing cytoxic antibodies.

6293 PURIFICATION OF TUMOR-SPECIFIC ANTIGEN OF MOUSE MAMMARY TUMOR.

(E.) Mitsui, H. (Natl. Cancer Cent. Res. Inst., Tokyo, Japan). *Gann Monogr Cancer Res* 16:155-166, 1974.

Unique methods for the detection, solubilization
and partial purification of a mouse mammary tumor-
specific antigen (MM antigen) were developed using
C3H/He mice. Resistance to the isografted tumor,
M2, originating from a spontaneous mammary tumor,
was induced by i.p. injection of MM2 sensitized
with MM2-immunized rabbit serum. After four weeks,
surviving mice were challenged with MM2, resulting
n a 70% survival rate; after the second challenge
there was a 100% survival rate without any signs
of tumor growth. The resistant mouse serum was
cytotoxic against MM2 cells (19S and 7S antibodies)
and was able to fix complement with MM2. The sur-
ace antigens were detected and characterized by
mmune adherence and immune adherence inhibition
ests. C3H/He mouse mammary tumors and Ehrlich
scites tumors absorbed MM2 antibodies, whereas
ther tumors and normal cells did not. Surface
ntigens of MM2 tumor cells reacting with tumor-
pecific antibodies were susceptible to treatment
ith organic solvents, some detergents and phospho-
ipase A and were fairly resistant to some proteoly-
ic enzymes. Deoxycholate extract of MM2 cells
eacted with the antibodies whereas the organic sol-
ents did not. The antigen was extracted with 0.2%
eoxycholate. After dialysis removal of deoxycholate,
he antigenic activity was recovered in a fluffy
ediment through centrifugation (105,000 *g* for 60
in) and was purified by treatment with 75% ethanol.
he antigenic activity (25% recovery) was found to
e purified 109-fold on a protein basis. Chemical
nalysis showed that it was composed of 11.7% pro-
ein, 83.3% lipid, 0.35% sugar and 3.9% RNA. The
urified preparation induced heightened resistance
gainst MM102 in syngeneic C3H/He mice. The results
uggest that the purified antigen was derived from
he plasma membrane of MM102 cells.

294 ACTIVATION OF HERPES SPECIFIC ANTIGENS AND INFECTIOUS VIRUS IN A HAMSTER CELL LINE
ANSFORMED BY HSV-2.

(E.) Vaczi, L. (Inst. Med.
crobiol., Univ. Debrecen, Hungary), I. Boldogh
d E. Gönczöl. *Arch Gesamte Virusforsch* 44(1):62-
, 1974.

established line of hamster embryonic fibroblast
lls transformed by herpes simplex virus type 2
SV-2), and derived from hamster tumors, contained HSV-specific intracellular and surface antigens in 2-5% of the cells but did not produce infectious virus. Arginine deprivation for four days resulted in induction of virus specific antigens in 70-75% of the cells and induction of surface antigens in 40%. Infectious virus could not be detected in arginine-deprived cultures. After three-day treatment with 5-iodo-2-deoxyuridine (IUDR, 40 µg/ml), 70-75% of the cells showed virus-specific intracellular fluorescence on the sixth day after treatment, and 45-50% exhibited cell membrane fluorescence on the ninth day. Infectious virus was also detected on the sixth day after IUDR treatment and yields reached 25-40 plaque-forming U/10^6 cells on the tenth day. No induction of antigens and infectious virus was observed when cultures were exposed simultaneously to IUDR and cytosine arabinoside hydrochloride (15 µg/ml). Thus, activation of HSV-2 transformed cells by IUDR requires DNA synthesis.

6295 AN ANTIBODY SANDWICH METHOD FOR THE RADIO-IMMUNOASSAY OF HUMAN α-FETOPROTEIN.

(E.) Pecec, M. (Inst. Oncol., Bucharest, Rumania) and N. Voiculetz. *Immunochemistry* 11(12):771-775, 1974.

In a new modification of a radio-immunoassay for human α-fetoprotein (α-FP), human (α-FP) serum was equilibrated with excess rabbit anti α-FP serum and the residue discarded. The residual rabbit anti-α-FP serum was then adsorbed onto BSA as a water-insoluble complex and was reacted with ^{125}I-labeled, purified goat anti-rabbit IgG antibody. The BSA complex was separated and counted, and the amount of original human α-FP was calculated from the radioactivity. The advantages of this method are: greater sensitivity, avoidance of labeling damages of the immune reagents, the capability of using the same labeled anti-IgG preparation for the analysis of several antigen-antibody systems and the radiolysis phenomena are less critical than in other radioimmunoassay procedures.

6296 RELATIONSHIP BETWEEN EPSTEIN-BARR VIRUS-DETERMINED COMPLEMENT-FIXING ANTIGEN AND NUCLEAR ANTIGEN DETECTED BY ANTICOMPLEMENT FLUORESCENCE.

(E.) Klein, G. (Dep. Tumor Biol., Karolinska Inst., Stockholm, Sweden) and V. Vonka. *J Natl Cancer Inst* 53(6):1645-1646, 1974.

A representative collection of human sera positive for Epstein-Barr virus (EBV) were tested for complement-fixing (CF) antibodies against the soluble (S) antigen contained in human lymphoblastoid lines carrying the EBV genome and for antibodies against an EBV-determined nuclear antigen (EBNA). As indicated by anti-complement fluorescence, there was a significant correlation (0.86) between the antibody titers to the CF(S) antigen and EBNA. A significantly lower correlation was found between the antibody titers to EBNA or CF (S) and anti-viral capsid antigen (VCA). The results indicate that antibodies to the CF (S) antigen and to EBNA may be identical; in testing this, cross-absorption tests may give the most conclusive results.

6297 SURFACE MEMBRANE ANTIGENS OF PY-3T3 MOUSE FIBROBLASTS.

(E.) Molinari, J. A. (Dep. Microbiol., Univ. Pittsburgh, Pa.), J. L. Ebersole and D. Platt. *Arch Gesamte Virusforsch* 46(3-4):229-237, 1974.

Female NIH Swiss and BALB/c mice were immunized i.c. with suspensions of PY-3T3 (polyoma virus-transformed 3T3 fibroblasts) and 3T3 plasma cell membranes. At seven days postimmunization, the mice were foot pad tested to detect delayed hypersensitive reactions. Measurement of the pad swelling 24 hr after challenge showed that 33 out of 40 of the NIH Swiss mice immunized with the PY-3T3 plasma membranes had delayed hypersensitive reactions, whereas none of the 19 BALB/c animals similarly immunized gave positive results. Mice of both strains immunized with normal 3T3 plasma membranes also failed to give positive reactions. C3H/HeJ mice were able to elicit a positive cell-mediated immune response to 3T3 fibroblast surface antigens, while NIH Swiss mice developed significant reactions only to surface antigenic determinants present on the polyoma-transformed cells. Thus, the response of the latter strain appeared to be directed toward viral-induced antigens which were absent from the normal 3T3 plasma membranes.

6298 ANTIGENICITY OF MURINE SKIN TUMORS INDUCED BY ULTRAVIOLET LIGHT.

(E.) Kripke, M. L. (Univ. Utah Coll. Med., Salt Lake City). *J Natl Cancer Inst* 53(5):1333-1336, 1974.

Primary skin tumors were induced in male and female C3H f/Sm and A/J mice by repeated exposure to UV light or repeated topical applications of benzo(a)pyrene (BP). The primary tumors were fragmented and transplanted s.c. into normal and immunosuppressed (ATX) syngeneic male recipients. The spindle cell tumors induced by UV light in C3H f/Sm and A/J mice were not transplantable in normal mice, although 73% of such tumors grew progressively in at least one ATX recipient. In contrast, primary spindle cell neoplasms induced by BP were readily transplantable in both normal and ATX syngeneic hosts. Of the UV-induced squamous cell carcinomas, 45% were transplantable in normal hosts and 85% grew progressively in at least one ATX recipient; there was no correlation between transplantability and dose of UV light or tumor latent period. Two UV-induced spindle cell tumors which grew in ATX recipients also grew in many mice immunosuppressed with rabbit antimouse-lymphocyte globulin but not in normal mice. Mice immunized s.c. with spindle cell tumor fragments and challenged with the same tumor were highly resistant to the development of the challenge implants. Mice immunized with normal skin or a different tumor from that used for challenge were equally susceptible to the growth of the challenge implants, indicating a lack of cross-reactivity among these tumors. No cross-reactivity was detected between two neoplasms arising in the same donor. The ability of mice to reject a UV-induced spindle cell implant was depressed immediately following whole-body X-irradiation, but was recovered about six weeks after irradiation. The failure of the spindle cell tumors to grow in normal syngeneic recipients appears mediated by an immune response against tumor-specific antigens.

6299 COMPARISON OF CARCINOEMBRYONIC ANTIGEN (CEA) METHODOLOGIES.

(E.) Fleisher, M. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.), E. Besenfelder, H. F. Oettgen and M. K. Schwartz. *Ann Clin Lab Sci* 4(5):357-362, 1974.

The three radioimmune assay procedures which have been used most often for the determination of carcinoembryonic antigen (CEA) differ primarily in the method of extraction of CEA from the plasma or serum and in the techniques used to separate free antigen from that which is antibody-bound. Method one involves $HClO_4$ extraction and $(NH_4)_2SO_4$ separation; method two involves $HClO_4$ extraction and sirconyl phosphate gel separation; and method three is a direct nonextraction method involving direct serum triple isotope-double antibody separation. All three methods were applied to the analysis of CEA in over 200 serum and plasma specimens. Using method two, the serum CEA levels were almost invariably higher (by an average of 3 ng/ml) than the plasma levels. In 83 assays comparing methods one and two, 73% concordance was obtained using a cut-off value of 5 ng/ml. Using a cut-off value of 15 ng/ml for method three, 62% concordance was found between methods one and three and 76% concordance was found between methods two and three. The precision of method two was found to be acceptable when a plasma pool was assayed 20 times over a two-day period by two technologists. This method appears to be a fair compromise between the lengthy and laborious procedure required for method one and the complex, highly sophisticated procedure required for method three. Also, unlike method three, neither method one nor two required special equipment or apparatus. The results of this study indicate that extraction of CEA from the serum or plasma is essential. Until sufficient information has been collected which will define the nature of CEA in various disease conditions, caution should be exercised in using CEA determinations as a diagnostic method.

6300 ANTIGENS OF CARCINOMA OF THE CERVIX UTERI: A STUDY BY MEANS OF IMMUNOFLUORESCENCE.

(E.) Nelson, D. S. (Dep. Bacteriol., Univ. Sydney, Australia). *Clin Exp Immunol* 16(1):53-62, 1974.

Tissues and sera from 30 patients with cervical carcinoma and from control patients were examined by means of indirect immunofluorescence staining for antibodies to cell constituents. In tests against autologous sera, 17 cervical carcinoma samples gave positive reactions with the epithelial portions' cytoplasmic constituents and seven of these were tested against three groups of cervical sections: (1) cervical carcinoma; (2) no known malignancy; (3) bodily carcinomas. A great majority of cervical carcinoma tissues had cytoplasmic antigens react with other sera, but some without carcinoma also reacted. Three cervical carcinoma and two normal cervical sera were also tested against the same three groups. The highest incidence of reactions was between cervical carcinoma sera and carcinoma tissue. Fewer reactions occurred between sera from the control groups and cervical carcinoma tissue. These reactions, together with absorption experiments, indicate that either or both of two common cytoplasmic antigens are present in all cervical carcinomas and in some non-malignant ervices. Seven cervical carcinoma sera were tested gainst sections of autologous tissue and then react- d with anti-IgA, anti-IgG and anti-IgM conjugates. our showed reactions with anti-IgG, one of which al- o reacted with anti-IgA and anti-IgM, and another ith only anti-IgM--indicating that there was no spe- ificity for other immunoglobins. In the preliminary urvey, several sera from patients with carcinoma had een noted to react with all nuclei in the tissue ections. A high incidence of antinuclear antibodies as found in patients with cervical carcinoma, cer- ical adenocarcinoma, or body, uterus or premalignant ervical lesions. It is suggested that this is a onsequence of increased cell damage and/or turnover nd not a consequence of malignant disease.

301 FURTHER INVESTIGATIONS OF THE EXPRESSION OF SV40-INDUCED SURFACE ANTIGENS SUBSEQUENT TO NZYMATIC TREATMENT OF TRANSFORMED CELLS. *(E.)* Rug- ieri, S. (Inst. Gen. Pathol., Univ. Florence, Italy), . J. Collins and P. H. Black. *FEBS Lett* 45(1):56-63, 974.

he extent of phospholipid hydrolysis in SV40-trans- ormed rabbit kidney cells (TRK-73) was measured af- er exposure of the cells to phospholipase C in the resence or absence of calcium. The cultures were abeled with (^{3}H)choline and (^{14}C)glycerol, and the elease of phosphoryl choline from the phospholipase -treated cells was measured by counting the ^{3}H label n the filtered supernatant. Treatment with phospho- ipase C, either in the presence or absence of cal- ium, caused an increase in the release of labeled osphoryl choline into the incubation medium. Di- lycerides were slightly increased in TKR-73 cells xposed to phospholipase C in the absence of cal- ium, but were increased approximately four-fold in ells treated with phospholipase C in the presence calcium. Measurement of the degree of fluores- ence staining of the SV40-associated antigen in TRK- cells exposed to phospholipase C with and without lcium demonstrated that an equivalent degree of aining was obtained in both the presence and ab- ence of calcium. The results indicate that: cal- ium is required for the hydrolysis of glycerophos- olipid molecules in TRK-73 cells treated with phos- olipase C; the release of phosphoryl choline is not accurate measure of phospholipase C hydrolysis of llular lecithin; release of phosphoryl choline from lls treated with phospholipase C in the absence of lcium was presumably effected through a mechanism her than phospholipid hydrolysis; and hydrolysis phospholipase C is probably not responsible for e exposure of SV40-induced surface antigens on the ll membrane of certain SV40-transformed rabbit and mster cells.

02 A RAPID MICROMETHOD FOR THE DETECTION OF MEMBRANE-ASSOCIATED ANTIGENS ON MONOLAYER LLS BY INDIRECT IMMUNOFLUORESCENCE. *(E.)* Sorg, (Dermatol. Clin., Univ. Munster, West Germany). *r J Immunol* 4(12):832-834, 1974.

direct membrane fluorescence technique requires latively large amounts of cells and reagents for handling and washing of the cells by centrifugation. A method using monolayer cells grown in microtiter plates, where all reactions and washing occur, was therefore developed. Antisera from malignant melanoma patients and healthy control donors were used. Serial dilutions starting from 1:4 to 1:32 were run in duplicates. Polyspecific HL-A antisera served as a positive control while serum from a systemic lupus erythematosus patient served as a viability control. Fluorescein conjugated antisera and goat anti-rabbit IgG were used at a 1:4 dilution throughout. Human melanoma cells were grown as monolayers. After reaching confluency cells were removed by trypsinization and seeded into microtiter plate wells (500 cells/well). After cell attachment plates were covered with 5 ml of complete medium and incubated. Tumors were washed once with 5 ml phosphate buffered saline (PBS) + 5% fetal bovine serum (FBS) and once with PBS + 2% FBS. After the second washing the buffer was aspirated and 10 μl of serum was added to each well. After 30 min at 37 C the cells were washed twice (same as previous washing), reacted with 10 μl fluoresceinated antiserum for 20 min at 37 C and washed twice again. The buffer was then removed and the plates were placed under a Universal fluorescence microscope in the regular position. Cells were then excited in incident light from a Philips CS 200 W-4 mercury lamp. Positive tests are noted by a bright continuous ring forming a contour around the cell membrane, while in negative tests the cell cannot be seen. The method allows for a quick evaluation and large scale testing with a minimum of reagents and cells.

6303 RESTRICTION OF ANTIGEN MOBILITY IN THE PLASMA MEMBRANES OF SOME CULTURED FIBROBLASTS. *(E.)* Edidin, M. (Biol. Dep., Johns Hopkins Univ., Baltimore, Md.) and A. Weiss. *Cold Spring Harbor Conf Cell Proliferation* 1:213-219, 1974.

Several well-ruffled cell types were examined for the ability of ligand-induced accumulation of cell surface proteins to predominate in one portion of the membrane ("capping"), believed to require both mobility of membrane molecules and ability of a cell to drive small aggregates of surface molecules and ligands in a constant direction (away from the cell's leading, ruffled edge). Cap formation was induced by coating cells [3T3, 3T12, SV40-transformed 3T3(SV 3T3)], and flat revertant SV3T3(FRSV-3T3), either in suspension or attached to a substrate, with anti-H-2 or anti-species antibodies at 0 C and then by reacting with fluorescent antiglobin. Despite extended treatment, 3T3 fibroblasts, WI-38 human fibroblasts and a normal mouse fibroblast strain failed to form caps. In contrast, SV3T3 cells capped readily though their capping was sensitive to antiserum dilution. The mobilities of H-2 and human species antigens were measured by fusing mouse and human cells and counting the number of heterokaryons with completely intermixed surface antigens and the number bearing segregated natigens at various times after fusion. The loss of segregated heterokaryons in populations formed from both contact inhibited and transformed cells was also measured. If one parent in a fusion does not cap, it was found that, regardless of the second cell type, heterokaryons will show some restriction of the mobility of surface antigens. The fusion of 3T3 with primary or secondary adult fibroblasts yields heterokaryons with an extremely low rate of mixing. In three experiments, most of the heterokaryons remained segregated at times when even the 3T3 x WI-38 heterokaryon population contained a considerable proportion of cells with completely intermixed surface antigens.

6304 ENHANCEMENT OF 3LL TUMOR GROWTH BY AUTOSENSITIZED T LYMPHOCYTES INDEPENDENT OF THE HOST LYMPHATIC SYSTEM.

(E.) Carnaud, C. (Weizmann Inst. Sci., Rehovoth, Israel), D. Ilfeld, Y. Levo and N. Trainin. *Int J Cancer* 14(2):168-175, 1974.

Autosensitized lymphocytes have been shown to mediate Lewis lung carcinoma (3LL tumor) cytotoxicity when tested *in vitro* and to promote tumor growth when tested *in vivo.* Whether these effects were exerted by the same lymphocytes or by different types of cells, and whether autoimmune lymphocyte promotion of tumor growth is mediated by a direct interaction with injected tumor cells or via the host immune system is reported. C57Bl/6 and C3H/eb mice were used with BN rat embryos serving as a fibroblast source for some experiments. Treated animals were whole body irradiated (750 r) and/or thymectomized by suction under nembutal anesthesia. *In vitro* measurement of cytotoxicity utilized the modified micro-assay, while *in vivo* analysis of tumor growth was done by daily inspection of footpads and palpatation of the subcutaneous tissue. When autoimmune spleen cells were injected with 3LL tumor cell suspensions, the time of tumor appearance was accelerated in both thymectomized plus irradiated and intact animals. When autosensitized lymphocytes were mixed with either 3LL tumor cells or fibrosarcoma cells and injected s.c. into intact C3H recipients a significant acceleration of tumor growth was noted in fibrosarcoma cases while 3LL tumor growth rate remained unaffected. *In vitro* exposure of B, T and B plus T cell populations obtained from bone marrow and thymocyte-reconstituted thymectomized, whole body irradiated C57Bl/6 mice to C57Bl/6 syngeneic fibroblasts and subsequent cytotoxic assay revealed T lymphocytic target damage to be significant while B cells were devoid of activity. When B and T cell populations were cultured on C57Bl/6 fibroblasts and then transferred with a 3LL tumor inoculum into immunologically impaired and intact C57Bl/6 mice, only autosensitized T cells conveyed tumor enhancement. These results indicate that under *in vivo* and *in vitro* conditions the effector cells committed against self are T lymphocytes. Autosensitized tumor enhancement was compared to the immune effects mediated by allosensitized lymphocytes, with no modification of tumor growth being seen in the allosensitized lymphocyte model. The tumor facilitation induced by autoimmune lymphocytes is thus indicated to be a T cell phenomenon independent of the host lymphoid system.

6305 A ROLE FOR SUPPRESSOR T CELLS IN IMMUNOLOGICAL ENHANCEMENT OF TUMOR GROWTH.

(E.) Kirkwood, J. M. (Sch. Med., Yale Univ., New Haven, Conn.) and R. K. Gershon. *Prog Exp Tumor Res* 19:157-164, 1974.

Evidence of suppressor functions of thymus-derived lymphocytes (T cells) in nontumor systems and in the immunological enhancement of tumor growth is reviewed. That T cells suppress the immune response in nontumor systems is indicated by the thymus dependence of some forms of immunological tolerance, the dependence of antigenic competition on T cell function, the possible role of T cells in preventing autoimmune disease, and the increased antibody responses to thymus-independent antigens seen after thymus deprivation. *In vivo* studies demonstrated the suppressive influence of specifically sensitized thymic T cells on the immune response to an allografted methylcholanthrene-induced sarcoma of BALB/c mice. Thymic T cells from A mice were sensitized by i.v. inoculation into lethally irradiated semiallogeneic hybrid (BALB/c x A) F_1 mice. Sensitized T cells were recovered from the spleens of the intermediate host and adoptively transferred back into A recipients together with sarcoma cells (5 x 10^6, s.c.). At a dose of 2.5 x 10^7, splenic T cells significantly enhanced tumor growth in comparison with controls receiving no T cells (or controls receiving identical numbers of nonspecifically sensitized T cells). No enhancement occurred with 1.0 x 10^6 splenic T cells, suggesting that suppressor T cell function is inhibited by stimulation above certain thresholds or is sensitive to autoinhibition by the same factors acting on other (effector) cells of the T-cell class, but at higher thresholds. Activation of suppressor T cells by antigen-antibody complexes is proposed as a single mechanism to explain the phenomenon of enhancement.

6306 LEUKAEMIC RETICULOENDOTHELIOSIS: A MORPHOLOGICAL AND IMMUNOLOGICAL STUDY OF FOUR CASES.

(E.) Haegert, D. G. (Dep. Pathol., Univ. Cambridge, England), J. C. Cawley, R. D. Collins, R. J. Flemans and J. L. Smith. *J Clin Pathol* 27(12):967-972, 1974.

The morphological and immunological studies of four cases of leukemic reticuloendotheliosis were conducted using patient cell preparations (bone marrow and peripheral blood) which were cultured with and without phytohemagglutinin (PHA) and were consequently assessed for transformation by morphological examination and light microscope autoradiography. The nature of the transformed cells was determined by sheep erythrocyte rosette and mixed antigobulin rosette reactions. Leucocyte preparations were examined by phase and interference light microscopy. Membrane fluorescence was performed on viable lymphocytes by direct method using fluorescein-labeled rabbit and sheep antisera to specific heavy and light chains. Characteristic hair like protrusions at the cell surface of the abnormal mononuclear cells were noted in suspension and in smear preparations. Distinctive cytoplasmic inclusions in many of the abnormal cells were seen in 2/4 cases. The

clusions were composed of parallel longitudinal
ibrils between which ribosome-like particles were
ound. In peripheral blood and bone marrow prepar-
ions, fine granular cytoplasmic PAS positivity was
all or nonexistent. A majority of the mononuclear
ells were shown to carry receptors for the Fc por-
ion of fixed IgG, while a smaller number had C_3 re-
ptors. A high incidence of surface IgG determi-
nts was seen in peripheral blood cultures, with
variable transformation rate of 2-32%. Immuno-
uorescent staining revealed a single light chain
terminant λ or κ to be present. The predominant
avy chain determinant was δ, with one case having
high percentage of surface μ determinants. Mor-
ological and cytochemical studies were inconclusive
to the nature of the abnormal mononuclear cells.
e low rate of transformation noted was consistent
th previous findings, and in addition showed that
e transformed cells were T cell type. These re-
lts indicate the hairy cell response to PHA to
analogous to that of the neoplastic cells in
ronic lymphatic leukemia. A monoclonal origin and
ngle light chain determinant of hairy cells is
ggested.

07 SURFACE ALTERATIONS AND MITOGENESIS IN LYMPHOCYTES. *(E.)* Edelman, G. M. (Rocke-
ller Univ., New York, N.Y.). *Cold Spring Harbor*
nf Cell Proliferation 1:357-377, 1974.

idence is presented for the modulation of cell
rface mobility by mitogenic lectins such as con-
navalin A (ConA). Native tetravalent ConA in-
ibited both patch and cap formation when added (> 5
/ml) to lymphocytes at 21 or 37 C prior to treat-
nt with anti-Ig. When cells were incubated with
nA at 4 C and then washed and brought to 37 C, the
ctin formed patches and caps with its own recep-
rs. ConA also inhibited the capping of antibody
ceptors induced by forming a sandwich of antigen
d antibody, prevented patching and capping by
tibodies to the θ antigen, and inhibited patch
d cap formation by its own receptors at 21 and 37
The antagonistic actions of ConA depended both
the temperature and the concentration. Although
wer molecules bound to splenic lymphocytes at 4 C
mpared with 37 C, ConA inhibition was apparent at
C (10^5-10^6 molecules bound/cell). Receptor mo-
lity was not inhibited by divalent ConA. With
travalent ConA, inhibition occurred at doses far
wer than those required to saturate all the ConA
nding sites. Inhibition was restored by antibod-
s to ConA and was reversed by colchicine and Vinca
kaloids. These observations are explained by an
chorage hypothesis assuming the existence of a
lchicine-binding assembly that is connected direct-
or indirectly to some of the cell surface recep-
s or the inner lamella of the cell membrane. If
nfirmed experimentally, the hypothesis would have
number of implications for cell-ligand and cell-
l interactions. It may explain how certain re-
ptors could remain relatively fixed on a membrane
le others move. It would help to explain avidity
ects in which clusters of receptors participate
specific interactions, whereas the dispersed re-
tors may not. Additionally, the hypothesis may
provide a connection between specific surface signals and alterations of cellular form.

6308 RECEPTOR SITES FOR AGGREGATED GAMMAGLOBULIN (AGG) ON LYMPHOCYTES IN LYMPHOPROLIFERATIVE DISEASES. *(E.)* Huber, C. (Med. Univ., Innsbruck, Austria), E. Dworzak, U. Fink, G. Michlmayr, H. Braunsteiner and H. Huber. *Br J Haematol* 27(4):643-654, 1974.

Normal and pathological lymphocytes were incubated with ^{125}I-labeled proteins and evaluated autoradiographically for receptor sites for aggregated gammaglobulin (AGG). Blood or lymph node lymphocytes were obtained from 18 patients with chronic lymphocytic leukemia (CLL), 17 with non-Hodgkin lymphoma, and 27 healthy controls. In 16/18 patients with CLL, the percentage of ^{125}I-AGG binding lymphocytes was markedly increased compared with the 15% average labeling in normal blood lymphocytes. The percentage of ^{125}I-AGG binding lymphocytes in CLL patients correlated significantly with the percentage of cells reacting with ^{125}I antilight chain antisera. With ^{125}I-AGG and with ^{125}I anti-Ig antisera, the staining intensity of leukemic lymphocytes was ofter reduced in comparison to normal controls. In 12/16 patients with non-Hodgkin lymphomas, the percentage of blood lymphocytes with receptor sites for AGG and the percentage of cells with Ig determinants were within the normal range. In 5/6 patients tested, more than 80% of the lymphoma cells stained with ^{125}I-AGG and a similar percentage stained with surface Ig of the IgM type. With few exceptions, patient lymphocytes forming rosettes with unsensitized sheep RBC did not bind ^{125}I-AGG. On density centrifugation, blood lymphocytes were depleted from cells forming rosettes with unsensitized sheep RBC; these B-cell-enriched suspensions contained more than 80% of lymphocytes binding ^{125}I-AGG. These results confirm the hypothesis of an abonormal B-cell proliferation in CLL patients and in some lymphomas.

6309 CHARACTERIZATION OF LYMPHOCYTE SUBPOPULATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA. *(E.)* Rowlands, D. T., Jr. (Sch. Med., Univ. Pennsylvania, Philadelphia), R. P. Daniele, P. C. Nowell and H. A. Wurzel. *Cancer* 34(6):1962-1970, 1974.

The B cell and T cell characteristics of the circulating lymphocytes were studied in 13 patients with chronic lymphocytic leukemia (CLL). Immunofluorescence of surface immunoglobulins and rosette formation (EAC) using sensitized sheep red blood cells were employed to detect B cells, while rosette formation (E) with untreated sheep red blood cells and *in vitro* phytohemagglutinin (PHA) stimulation and cytogenetics were used to study T cells. The findings generally confirm the conclusions of others that CLL usually represents a proliferative disorder of B cells, probably clonal in nature. In 11 cases, the predominant cell surface immunoglobulin was IgM; in one case it was IgG and in one case there was only a slight elevation in the incidence of IgM positive cells. In general, EAC rosette studies confirmed the observations made using immunofluorescence, but in one case this correlation was apparent only after the incidence of EAC rosettes was increased by pretreatment of the CLL cells with 2-mercaptoethanol. In another case, the incidence of EAC rosette-forming cells significantly exceeded the incidence of immunofluorescent cells, perhaps suggesting a variable expression of different surface markers on neoplastic cells in CLL. E rosette studies and PHA cultures indicated a decreased proportion of T cells in the peripheral blood of the patients, but suggested that the absolute number of T cells was not reduced. Kinetic and cytogenetic studies with PHA cultures further demonstrated no qualitative abnormality in the circulating T cells in this disease, supporting the view that they represent a normal population diluted in a large pool of neoplastic B cells.

6310 THE ROLE OF NUCLEAR-MATERIAL IN THE TRANSFER OF IMMUNOLOGICAL INFORMATION. II. THE MODE OF ACTION OF NUCLEAR MITOGEN. *(E.)* Lewis, C. M. (Charing Cross Hosp. Med. Sch., London, England) and G. D. Pegrum. *Immunology* 27(5):929-939, 1974.

In this study of the effect of lymphocytes stimulated by phytohemagglutinin (PHA) on DNA synthesis of target B or T cells, it is demonstrated that only the nuclei of PHA-stimulated lymphocytes are required. After incubation of lymphocytes with ^{125}I-labeled PHA, label accumulated in the RNA fraction of the nucleus and this nuclear label could be transferred to fresh lymphocyte cytoplasm but not to nuclei. Anti-sera to PHA or concanavalin A (Con A) inactivated the mitogenic activity of the native lectins but higher concentrations were needed to inactivate the transformed nuclei. Similary, α-methyl mannoside and *N*-acetyl-D-galactosamine which competitively inhibit Con A and PHA were required in massive concentrations to inactivate lectin-transformed nuclei. Further experiments with antisera supported the hypothesis that the blastogenic effects of lectin-transformed nuclei were at least partly due to transfer of the RNA-complexed mitogen either within or bound to the nuclear membrane.

6311 SELECTIVE ACTIVATION OF T LYMPHOCYTES. ITS POSSIBLE APPLICATION IN THE IMMUNOMANIPULATION OF TUMOURS. *(E.)* Miller, J. F. A. P. (Royal Melbourne Hosp., Australia). *Recent Results Cancer Res* 47:5-11, 1974.

A method by which thymus cells can be "activated" against antigenic tumor cells is described. Thymus cells from CBA mice were injected into heavily irradiated (800 R) (CBA X C57BL)F_1 and (CBA X BALB/c)F_1 hosts, thus providing a source of antigens identical to those present on the DBA/2 mastocytoma selected for study. The thymus cells proliferated and produced a progeny of circulating lymphocytes which were immunologically competent and highly specialized in the sense that they could react only to the antigens which initially provoked their formation. These activated lymphocytes (T. TDL) were highly effective in protecting T cell-deprived mice from the growth of inoculated mastocytoma cells and in lysing the tumor cells *in vitro*. The T. TDL, as a pure population of killer lymphocytes, offer distinct advantages over killer cells obtained by other methods in that they show a high viability (98%) and a low degree of contamination by other cell types (0-5%). However, before immunomanipulation can become a practical reality in clinical medicine, it will be necessary to obtain more detailed knowledge concerning the mechanism by which T and B lymphocytes respond to tumor-specific antigens.

6312 HUMAN PERIPHERAL LYMPHOCYTES AND CANCER: IN VITRO STUDIES ON THE BASIC PROTEIN, HISTONE F2A1 FRACTION. *(E.)* Fish, R. G. (Tenovus Labs., Velindre Hosp., Cardiff, Wales), J. A. V. Pritchard and T. J. Deeley. *Br J Cancer* 30(3):222-230, 1974.

The interaction of the highly purified basic protein "antigen", calf thymus histone F2A1 fraction, with peripheral lymphocytes isolated from untreated cancer patients and healthy volunteers was studied. The basic protein remaining in the supernatant fluid after interaction with low concentrations of lymphocytes was analyzed by SDS polyacrylamide gel electrophoresis. Using lymphocyte preparations from the patients with malignant disease, a new radioactive peak of molecular weight smaller than the original histone F2A1 fraction appeared. This low molecular weight radioactive material was largely or completely absent when lymphocytes from the normal subjects were used. The appearance of the low molecular weight radioactive peak was dependent on the lymphocyte concentration. Although the isolated cell preparations from both groups of subjects consisted mainly of lymphocytes, the peripheral blood from the cancer patients was contaminated to a greater degree with other blood components. The differences in degradation of the histone F2A1 fraction may be due to a difference between the two lymphocyte preparations in respect to proteolytic activity. In this study conditions for the interaction of basic protein with lymphocyte preparations are identical to those used for the macrophage electrophoretic mobility test. It is considered possible that partial degradation of other basic proteins, e.g. encephalitogenic factor, by proteolytic activity of cells from cancer patients could produce peptide fragments which in turn could act as macrophage slowing factors.

6313 MECHANISM OF CYTOLYSIS BY THYMUS-DERIVED LYMPHOCYTES. *(E.)* Henney, C. S. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.). *Prog Exp Tumor Res* 19:203-216, 1974.

A scheme for the lytic process mediated by thymus-derived lymphocytes (T cells) from immune animals is described. The model system measured cytolysis by incubating cell suspensions from the spleens of alloimmunized C57BL/6 mice with ^{51}Cr-labeled mastocytoma cells. Cytolysis was evaluated by measuring the amount of ^{51}Cr released from target cells and correcting the value according to the amount of ^{51}Cr released in the presence of normal C57BL/6 spleen cells. There was no evidence of other cell types and complement has no role in the lysis, which was inhibited by cytochalasin B (the degree of inhibition was dependent of the amount of cytochalan B and the stage of its administration). The use pactamycin and emetine (inhibitors of initiation d elongation, resp.) demonstrated the necessity continued protein synthesis for cytolysis. An verse relationship was noted between the amount intracellular cAMP and cytolysis which may be diated by interference with cellular secretory ents. Various inhibitors of cytolysis have been own to exert their effects at different stages. e relative sequence for these inhibitors is as llows: cytochalasin B, pactamycin, cAMP-active ugs, colchicine, and EDTA. The specificity of ese inhibitors in more well-defined systems has en used to infer their role in the cytolytic path- y. The hypothesis views T-cell-mediated cytolysis a secretory event in which a soluble mediator is leased from the lymphocyte to target cell after tigen-lymphocyte interaction and effects target ll lysis. Proof of this mechanism would help in tablishing protection against tumor growth.

14 *IN VITRO* COLONY-FORMING CELLS AND COLONY STIMULATING FACTOR IN CHRONIC GRANULO- TIC LEUKAEMIA. *(E.)* Goldman, J. M. (Royal Post- ad. Med. Sch., London, England), K. H. Th'Ng and M. Lowenthol. *Br J Cancer* 30(1):1-12, 1974.

ood and bone marrow samples obtained from 23 pa- ents with chronic granulocytic leukemia (CGL), 1 Ph^1 positive, were studied using agar cell cul- res. Bone marrow specimens taken from untreated L patients contained three times the number of lony forming cells (CFC) than those from normal ntrols. Peripheral blood samples from untreated L patients contained even higher numbers of CFCs ich were related to total leukocyte count. Peri- eral blood, but not bone marrow numbers of CFCs, s reduced by cytotoxic drug treatment. Peripher- blood leukocytes from untreated CGL patients were poor source of colony stimulating factor (CSF), d it is suggested that this is not just due to a lative scarcity of monocytes in this preparation. rmal CSF activity was seen in leukocytes obtained om treated patients and was associated with the nocytic cell component. It is suggested that cy- toxic drug treatment may lead to a replacement of ukemic monocytes by normal monocytes, or that drug eatment may in some way remove an inhibitory fac- r which suppresses CSF formation by normal mono- tes.

6 5 MODULATION OF CELL PROLIFERATION BY MACRO- PHAGES: A POSSIBLE FUNCTION APART FROM OTOXIC TUMOUR REJECTION. *(E.)* Keller, R. (Im- obiol. Res. Group, Univ. Zurich, Switzerland). *J Cancer* 30(5):401-415, 1974.

in vitro interaction between activated, nonim- e macrophages (AM) and a variety of syngeneic, ogenic, or xenogeneic normal and malignant tar- cell lines was assessed by evaluating target l proliferation, viability, and morphology. The ostatic macrophage effect was similarly potent inst syngeneic, allogeneic, or xenogeneic tumor targets whether autochthonous or induced by viruses or carcinogens; proliferation of rapidly replicating cell lines was blocked by the presence of AM in an effector/target cell ratio of 10:1. Moreover, proliferation of rapidly replicating normal cells was blocked almost as completely as that of neoplastic targets. Only in slowly proliferating cells was the inhibitory effect of AM not detectable. A marked diminution in target cell proliferation also occurred in targets grown in suspension, indicating that the macrophage effect may be mediated by a soluble product of AM. The finding of clear differences in the proliferation inhibition of slow proliferating normal and neoplastic cells suggested that proliferation *per se* may not fully reflect the consequences of the macrophage/target cell interaction. This was confirmed when viability and morphology were studied; viability was virtually unaffected in normal targets while neoplastic cells were killed. These results suggest that AM affects targets in strikingly different ways. Inhibition of proliferation could be an important homeostatic regulatory macrophage function that affects every replicating cell. Cytocidal killing of targets, on the other hand, is achieved only on neoplastic cells.

6316 MYELOMATOUS PARAPROTEINEMIA OF THE IgA OR IgG AND BLOOD LIPIDS. *(Fr.)* Clerc, M. (Univ. Hosp. Cent., Abidjan, Ivory Coast), A. Triquet and J. Martine. *Bull Soc Pathol Exot* 67(1): 101-108, 1974.

The correlation of hypo or hyper lipidemia with myeloma was investigated in an African patient population: serum cholesterol, lipids and immunoglobulin A (IgA) and G (IgG) fractions of 11 African myeloma patients was described. The serum levels of IgG in these patients were three times higher than IgA levels. Immunoelectrophoresis demonstrated six types of paraproteinemia with fast and slow γ or β IgA or IgG bands, and serum lipids were depressed (mostly due to decreased cholesterol). Hypolipidemia and hypocholesterolemia of these myeloma patients are considered as being related to this malignant condition in view of the high incidence of these blood abnormalities in Africans.

6317 TUMOR INCIDENCE AND IMMUNOSUPPRESSION IN MALARIA-INFECTED MICE. *(E.)* Hargis, B. J. (Child. Cancer Res. Found., Boston, Mass.) and S. Malkiel. *Fed Proc* 33(3):770, 1974.

6318 IMMUNODEPRESSION OF NORMAL MOUSE SPLEEN CELLS BY FRIEND VIRUS (FV) LEUKEMIC CELLS *IN VITRO.* *(E.)* Weislow, O. S. (Thomas Jefferson Univ., Philadelphia, Pa.) and E. F. Wheelock. *Fed Proc* 33(3):770, 1974.

6319 EXPRESSION OF Thy-1 AND TLa ON MURINE LEUKEMIC LYMPHOBLASTS GROWN IN VARIOUS TYPES OF SUSPENSION CULTURE APPARATUS. *(E.)* Zwerner, R. K. (Univ. Alabama, Birmingham), T. H. Stanton, P. F. Weinheimer and R. T. Acton. *Fed Proc* 33(3):777, 1974.

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6322 THE IMMUNOLOGIC STATUS OF BDF_1 MICE RESISTANT TO REPEATED TUMOR CHALLENGE. *(E.)* Barker, A. D. (Battelle's Columbus Lab., Ohio), A. J. Dennis and R. M. Folk. *Fed Proc* 33(3):781, 1974.

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6324 TUMOR-INDUCED ANTILYMPHOCYTIC AUTOIMMUNITY IN L1210-BEARING MICE. *(E.)* Devlin, R. G. (Mead Johnson Co., Evansville, Indiana), L. E. Baugh, J. D. McCurdy and P. E. Baronowsky. *Fed Proc* 33(3):769, 1974.

6325 ENHANCEMENT OF MURINE LEUKEMIA BY MYCOPLASMAS: SUPPRESSION OF CELL-MEDIATED IMMUNITY BUT NOT HUMORAL IMMUNITY. *(E.)* Eckner, R. J. (Roswell Park Mem. Inst., Buffalo, N.Y.), T. Han and V. Kumar. *Fed Proc* 33(3):769, 1974.

6326 AN IMMUNOSUPPRESSIVE PEPTIDE FRACTION IN CANCER SERUM. *(E.)* Constantian, M. B. (Boston Univ., Mass.), R. B. Nimberg, A. H. Glasgow, J. O. Menzoian, K. Schmid and J. A. Mannick. *Fed Proc* 33(3):770, 1974.

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349 IMMUNOSUPPRESSION IN MICE FOLLOWING 334C MURINE LEUKEMIA VIRUS INFECTION. *(E.)* henk, P. J. (Roswell Park Mem. Inst., Buffalo, Y.) and R. F. Buffett. *Fed Proc* 33(3):619, 1974.

50 EFFECT OF IMMUNOSUPPRESSION ON GENETIC RESISTANCE TO MURINE INFLUENZAL ENCEPHALITIS. .) Fiske, R. A. (Dep. Pathol., Univ. Florida, inesville) and P. A. Klein. *Fed Proc* 33(3):619, 1974.

6351 DIFFERENTIAL SUSCEPTIBILITY BETWEEN TUMORIGENIC AND NON-TUMORIGENIC PAIRED CELL LINES TO BCG ACTIVATED MOUSE PERITONEAL MACROPHAGES. *(E.)* Tucker, R. (Natl. Inst. Health, Bethesda, Md.), M. Meltzer and K. Sanford. *Fed Proc* 33(3):615, 1974.

6352 CROSS REACTING CELLULAR HYPERSENSITIVITY AGAINST HUMAN *IN SITU* BREAST CANCER AND MURINE MAMMARY TUMOR VIRUS. *(E.)* Black, M. M. (New York Med. Coll., N.Y.), D. H. Moore, B. Shore, R. E. Zachrau and H. P. Leis, Jr. *Fed Proc* 33(3):609, 1974.

6353 B LYMPHOCYTES IN ACUTE LYMPHOBLASTIC LEUKEMIA: THE EFFECTS OF RADIATION AND CHEMOTHERAPY. *(E.)* Gajl-Peczalska, K. J. (Univ. Minnesota, Minneapolis), J. Zusman, J. H. Kersey and M. E. Nesbit. *Fed Proc* 33(3):610, 1974.

6354 *LIMULUS* AGGLUTININ DIFFERENTIATION OF HUMAN LEUKEMIC FROM NORMAL LYMPHOCYTES. *(E.)* Cohen, E. (Roswell Park Mem. Inst., Buffalo, N.Y.) and L. Pliss. *Fed Proc* 33(3):610, 1974.

6355 CARCINOMA OF THE ESOPHAGUS: ITS EFFECT ON THE ISOANTIGENS A, B AND H OF THE EPITHELIUM. *(E.)* Davidsohn, I. (Univ. Health Sci./Chicago Med. Sch., Ill.) and P. Lill. *Fed Proc* 33(3):609, 1974.

6356 A COMPARISON OF ENZYME-ACTIVE MEMBRANE ANTIGENS FROM TWO DIFFERENT DMAB-INDUCED HEPATOMAS WITH THOSE OF ADULT AND FETAL RAT LIVER. *(E.)* Raftell, M. (Wenner-Gren Inst., Univ. Stockholm, Sweden), F. Blomberg and P. Perlmann. *Conf Embryonic Fetal Antigens Cancer (Knoxville) November 4-7, 1973* 3:22, 1973.

6357 EFFECT OF ANTI-EMBRYO IMMUNIZATION ON METHYLCHOLANTHRENE-INDUCED SARCOMA GROWTH IN BALB/c MICE. *(E.)* Parmiani, G. (Natl. Inst. Stud. Cure Tumors, Milan, Italy) and R. Lembo. *Int J Cancer* 14(4):555-564, 1974.

6358 INCIDENCE OF CYTOTOXIC ANTIBODIES IN SERUM FROM NEOPLASIA PATIENTS. *(E.)* Abeatici, S. (2nd Sch. Gen. Clin. Surg. Surg. Ther., Univ. Turin, Italy) and A. M. Raso. *Panminerva Med* 16(7/8): 203-206, 1974.

6359 RECENT ELECTRON MICROSCOPIC AND IMMUNOLOGIC STUDIES ON BOVINE CELL CULTURES CONTAINING C-TYPE VIRUSES. *(E.)* Ferrer, J. F. (Sch. Vet. Med., Univ. Pennsylvania, New Bolton Cent., Kennett Square), L. Avila and N. D. Stock. *Bibl Haematol* (39):206-214, 1974.

6360 CELL-MEDIATED IMMUNITY IN ALLOGENEIC AND TUMOR-SYSTEMS: NATURE OF THE CELLS INVOLVED IN KILLING TARGET CELLS. *(E.)* Leclerc, J. C. (Saint Louis Hosp., Paris, France), J. P. Levy, E. Gomard, F. Plata and F. M. Kourilsky. *Recent Results Cancer Res* 47:122-126, 1974.

6361 ANTIBODY SECRETION BY MURINE MYELOMA MOPC-315 CULTURED *IN VITRO* AND STUDY AT THE INDIVIDUAL CELL LEVEL. *(Fr.)* Weyer, J. (Dep. Cell Immunol., Pasteur Inst., Paris, France) and A.-E. Bussard. *Ann Immunol [C] (Paris)* 125(6):947-958, 1974.

6362 SPECIFICITY OF HUMAN SERUM ANTIBODIES TO INTRACYTOPLASMIC A TYPE PARTICLES FROM MURINE MAMMARY CARCINOMA. *(Ger.)* Muller, M. (Carl Gustav Carus Med. Acad., Dresden, E. Germany), S. Zotter, C. Kemmer, B. A. Johannsen and H. Grossmann. *Arch Geschwulstforsch* 44(4):328-342, 1974.

6363 IMMUNOCHEMICAL STUDIES ON SERUM FROM TUMOR PATIENTS. *(Ger.)* Rimbaut, C. (Inst. Sci. Res. Cancer, Villejuif, France), G. P. Tilz and D. Buffe. *Med Klin* 69(35):1399-1401, 1974.

6364 IMMUNOCHEMICAL IDENTIFICATION OF β_1-GLOBULIN IN THE "PREGNANCY ZONE" IN SERUM FROM PATIENTS WITH TROPHOBLASTIC TUMORS. *(Fr.)* Tatarinov, Iu. S. (N. I. Pirogov 2nd Inst. Med., Moscow, USSR), N. V. Mesniankina, D. M. Nikulina, L. A. Novikova, B. O. Toloknov and D. M. Falaleeva. *Int J Cancer* 14(4):548-554, 1974.

See also:

* (Rev); 6007, 6022, 6029, 6030, 6031, 6032, 6033, 6034, 6035, 6036, 6037, 6039, 6056, 6063, 6065, 6066, 6080, 6081
* (Chem): 6111, 6132, 6133, 6136
* (Viral): 6191, 6197, 6200, 6210
* (Path): 6380, 6395, 6412, 6416

6365 A PRESUMABLY UNIQUE INTRACRANIAL TUMOUR ('ELASTOMA'). *(E.)* Ebels, E. J. (Dep. Pathol., Univ. Groningen, Netherlands) and K. G. Go. *Clin Neurol Neurosurg* 77(1):20-25, 1974.

An intracranial tumor in a 42-yr-old man is described. Histologically, the tumor showed a rather heterogeneous picture with marked variation in cellularity. In the cellular areas, groups of cells with round to ovoid nuclei and little cytoplasm were surrounded by a thin vascular stroma; some mitotic figures were present in these areas. Other areas showed a marked preponderance of interstitium. The most conspicous and consistent feature was the presence throughout the tumor of elastin deposits. Many of these were globular in shape, others were linear or more irregularly shaped. The globular deposits were somewhat larger than the cell nuclei. The presence of the deposits in the cellular, interstitium-poor areas cannot be interpreted as broken-up preexisting fibers or degenerating collagen. They appear to constitute an essential aspect of the neoplastic lesion, present from the earliest stages of development. This tumor therefore differs essentially from the elastofibroma dorsi. Whether it originated in the brain or in the meninges could not be established.

6366 THE ANALYSIS OF TUMOR TRANSFORMATION OF TISSUES. IV. ON CHROMOSOME ABERRATIONS DURING BLASTOGENESIS. *(Rus.)* Salyamon, L. S. (Inst. Cytol. U.S.S.R. Acad. Sci., Leningrad) and L. A. Tchernina. *Tsitologiia* 16(10):1275-1283, 1974.

This was the continuation of a previous study in which the present authors observed that application of benzpyrene did not produce any significant effect on the frequency of chromosome aberrations in experimental animals. Only when ionizing radiation was used subsequent to application of benzpyrene was such an effect observed. In the present study, 7,12-dimethylbenzanthracene (DMBA) was used as the experimental carcinogen. Mouse cochlea were daubed with 0.05 and 0.1% DMBA in acetone or mineral (also vegetable) oil solutions and after a week or two were exposed to ionizing radiation. Results showed that using such a technique, the frequency of chromosome aberrations in the epidermis of mouse ears was higher than in control mice exposed only to ionizing radiation. The effect of DMBA in that respect was stronger than that shown by benzpyrene.

6367 DERMATOGLYPHS IN LEUKAEMIA. A STUDY OF 212 PATIENTS. *(E.)* Ventruto, V. (United Hosp., Naples, Italy), L. Sebastio, B. Festa, L. Farina and F. Caputo. *Boll Soc Ital Biol Sper* 50(18):1505-1508, 1974.

Fingerprints and palmar configurations were obtained from 212 patients with different types of leukemia and compared to those of subjects with no evidence of malignancy. The patients were subdivided according to the type of leukemia and were analyzed separately for sex. Analysis of the four main digital patterns (whorls, ulnar loops, radial loops and arches) in the patients and control groups, showed differences statistically significant in acute leukemias for both male and female adult patients, in females with chronic lymphatic leukemia, as well as in females with chronic myelogenous leukemia. The Sydney line was significantly increased in adult patients with acute leukemia and in male children with acute leukemia. No substantial differences in order to a-b ridge count and the atd angle were observed. These results agree with previous studies made in different geographic areas. It seems that each subcategory of leukemia has a typical pattern type of finger, such as an increased incidence of arches in females with chronic lymphatic leukemia, and of radial loops in males with the same disease; for both sexes there is an increase in whorls and a decrease in ulnar loops in chronic myelogenous leukemia. The same pattern has been observed in children with acute leukemia. An increased incidence of Sydney lines was observed in all studies of children with acute leukemia. These results account for the utility of dermatoglyphic patterns, which may reflect a genetic constitution predisposing to the enhancement of leukemia.

6368 THE CELL CYCLE AND THE NEOPLASTIC TRANSFORMATION. *(E.)* Lin, J. C. (Temple Univ. Sch. Med., Philadelphia, Pa.) and R. Baserga. In: *Developmental Aspects of Carcinogenesis and Immunity* (New York: Academic Press, 1974) pp. 65-77.

The synthesis of nonhistone chromosomal proteins and other nuclear proteins was studied in Simian virus 40-transformed human diploid fibroblasts (2RA). Cells grown for nine days in isoleucine-deficient modified Eagle medium (MEM) showed reduced ability to incorporate ^{3}H-thymidine into DNA but were stimulated to proliferate again by complete fresh MEM + 5% calf serum. At nine hr after stimulation, the incorporation of ^{3}H-thymidine was 20-fold above control values. Incorporation of ^{3}H-tryptophan into total cellular proteins reached a peak at 12 hr, while the peak incorporation of ^{3}H-tryptophan into chromatin and nonhistone chromosomal proteins was at six hr. These results suggest some fundamental differences in the prereplicative phase of 2RA and normal fibroblasts (WI-38). In WI-38 cells, the synthesis of nonhistone chromosomal proteins increases within 30 min of nutritional stimulation, the template activity of isolated chromatin at one hr, and the ability of cells to take up nonmetabolizable amino acids at three hr. This is followed by a second round of increased synthesis of nonhistone chromosomal proteins, a peak in the synthesis of ribosomal RNA at eight hr after stimulation, replication of DNA and histones at 12-15 hr, and, finally, mitosis. The first round of nonhistone chromosomal protein synthesis and the increase in chromatin template activity are actinomycin D-insensitive, while all other steps are actinomycin D-sensitive. In stationary 2RA cells the stimulus causes a prompt increase in the membrane transport function of the 2RA cells, followed by the other biochemical events described for the WI-38 cells. The question of why WI-38 cells, but not 2RA cells, are capable of entering the G_0

phase is discussed. It is postulated that the absence or marked decrease of certain nonhistone chromosomal proteins in 2RA cells causes them to remain in a G_1 phase after reaching a stationary phase. (45 references)

6369 CHROMOSOMAL ANOMALIES IN BENIGN AND PREMALIGNANT BREAST LESIONS. *(E.)* Kallenberger, A. (Zahnarztliches Univ. Inst., Basel, Switzerland). *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:171-179, 1974.

Short-term tissue cultures of human breast explants arrested with colchicine were used to provide mitotic figures for chromosomal analysis. In explants from 99 patients, including several premalignant or benign tumorous breast tissue (96 cases) plus preinvasive breast cancer (3 cases), only seven of the explants could not be cultured; 51/92 were chromosome-counted and the remainder karyotyped. Of the 48 premalignant and benign lesions (adenosis, simple cystic disease, proliferating cystic disease, cystic disease associated with cancer, fibroadenoma) 19/48 were hyperdiploid (range 2.1-2.5 c). All three preinvasive breast cancers were hyperdiploid. From karyotype analyses, 18/39 benign/premalignant explants had chromosome anomalies and 2/2 had preinvasive breast cancer. Euploidy was proportional to the proliferating state, being highest in adenosis (83-89%) and lowest in preinvasive cancer (17.6%). Out of a total of 300 karyotype analyses, 43 were anomalous, the most frequent anomaly (32/43) being large subtelocentric chromosomes. It is concluded that these studies support the view that cystic lesions and possibly fibroadenoma with epithelial proliferation share chromosome anomalies with mammary cancer.

6370 STUDIES ON RELATIONSHIP BETWEEN EPITHELIAL DYSPLASIA AND CARCINOMA OF THE ESOPHAGUS. *(E.)* Anonymous. (Esophageal Carcinoma Res. Coord. Groups, Chinese Acad. Med. Sci. and Honan Province). *Chin Med J* (11):190, 1974.

The relationship between epithelial dysplasia and carcinoma of the esophagus was investigated in four studies on patients in China. Among 7212 inhabitants over 30 yr of age in Linhsien county, the incidence of both esophageal carcinoma and dysplasia was higher in the north, indicating that there may be a relationship between the two. Among 21,581 rural inhabitants over 30 yr of age in the regions high in incidence of esophageal carcinoma, the incidences of esophageal dysplasia and carcinoma rose with age. Incidence of marked dysplasia before 50 yr of age was higher than that of carcinoma, indicating that cancerous changes of esophageal epithelium ran a continuous process from mild dysplasia through marked esophageal dysplasia, 26.6% progressed to carcinoma, a frequency 140 times that in the normal group. Of 105 cases of mild esophageal dysplasia, 16 cases progressed to marked dysplasia. Histological examinations of 67 cases showed that the process from simple hyperplasia to atypical hyperplasia and carcinoma *in situ* was a continuous one. Since in most cases carcinoma *in situ* was surrounded by atypical hyperplasia of the epithelia, cancerous changes were considered to arise from atypical hyperplasia. The results of the studies show that marked dysplasia can reasonably be regarded as a precancerous stage.

6371 CANCERS OF THE LARYNX AND HYPOPHARYNX. I SURGICAL PATHOLOGY. *(E.)* Nirmala, P. (St. John's Med. Coll., Bangalore, India), J. A. Thomas and S. N. Kothare. *J Postgrad Med* 20(4):153-159, 1974.

Cancers of the larynx and hypopharynx were diagnosed in 53 (5.7%) of 922 cancer patients seen at an Indian hospital over a seven-yr period. Most of the 53 patients (48 men and five women) were aged 41-50 yr. Hoarseness was the main symptom in 41 patients and 16 of these patients had no vocal cord involvement. Twenty-seven patients had laryngeal lesions of which seven were supraglottic; the hypopharyngeal area was involved in 19 with piriform fossa lesions notable in 13. All tumors were squamous cell carcinomas (64.1% grade I-II) and all showed adjacent mucosal ulceration with numerous inflammatory cells. In seven patients, the primary tumor had metastasized to the lymph nodes; 50% of the metastases were from tumor of the supraglottic and glottic areas and the other 50% were from tumors of the piriform fossa. The marked superficial ulceration and preponderance of grade I-II squamous cell carcinomas, coupled with similar incidences of cancer of the mouth (6.6%) and esophagus (7.2%), indicated a probable common predisposing factor. Since bètel leaf-chewing and the smoking of an indigenous cigarette (beedi) are common practices in India, the factor was probably the constant discharge of infective and carcinogentic material into the pharyngeal region from the oral cavity.

6372 GASTRIC LESION OF FAMILIAL POLYPOSIS COLI. *(E.)* Utsunomiya, J. (Tokyo Med. Dent. Univ., Japan), T. Maki, T. Iwama, Y. Matsunaga, T. Ichikawa, T. Shimomura, E. Hamaguchi and N. Aoki. *Cancer* 34(3):745-754, 1974.

Whether or not gastric carcinoma is due to malignant transformation of gastric polyps is not yet known, although the frequent occurrence of these polyps in cases of familial polyposis might indicate that some factors acting on the mucosa of the large intestine are also acting on the gastric mucosa. Familial polyposis coli is characterized by innumerable adenomas diffusely distributed over the large intestine, with an occurrence governed by simple Mendelian inheritance. Fifteen cases of this disease in 6 different unselected families are reported on in this study. Roentgenogram and endoscopic examinations were carried out in 12 cases, the resected stomach was examined in two cases, and autopsy material was used in two. Elevated lesions were found in the stomachs of ten patients (66.7%), which was a much higher incidence than in the results of a nationwide survey of this disease in Japan (10%). The authors suggest that this disease is not necessarily localized, and that the absence of gastric polyps in other case reports is probably due to insufficient examination of the stomach. The possibility that these findings are influenced by an environmental factor in Japan is also considered.

6373 ENZYMOLOGICAL CHANGES IN ABNORMAL DIFFERENTIATION: INTESTINAL METAPLASIA IN HUMAN GASTRIC MUCOSA: A POSSIBLE PRECANCEROUS CHANGE. *(E.)* Sugimura, T. (Natl. Cancer Cent. Res. Inst., Tokyo, Japan), T. Kawachi, K. Kogure, A. Tokunaga, N. Tanaka, K. Sasajima, Y. Koyama, T. Hirota and R. Sano. *Proc Int Symp Princess Takamatsu Cancer Res Fund,* 4th, 1973, pp. 251-261.

Sucrase activity in gastric mucosa was investigated by the Tes-Tape method in 117 stomachs resected from patients with gastric carcinoma. All carcinomas were negative for sucrase, but in 96 cases the tissue surrounding the carcinoma gave a positive reaction for sucrase. To further investigate the intestinal metaplasia (appearance of intestinal-type epithelial cells in gastric mucosa) revealed by the sucrase test, tests for four kinds of intestine-specific enzymes were made on each specimen. In addition, thick sections of specimens were examined for the presence of goblet cells and Paneth cells. Two types of intestinal metaplasia were found. One type (A) is characterized by the presence of sucrase, maltase, trehalase, and alkaline phosphatase plus goblet cells and Paneth cells; the second type (B) is characterized by the presence of sucrase, maltase, and goblet cells and absence of trehalase, alkaline phosphatase, and Paneth cells. One-third of the specimens showed type A intestinal metaplasia; 1/3, type B; and 1/3, both types. Thus the frequencies of cancers originating from types A and B were not significantly different. Whether intestinal metaplasia is obligatory in malignant transformation or whether the stomach epithelium can be converted to a metastable, unidentified state from which both cancer and intestinal metaplasia may arise remains to be determined.

6374 AN EXPERIMENTAL STUDY ON ATYPICAL EPITHELIAL PROLIFERATIONS ASSOCIATED WITH CHRONIC ULCERATION OF THE STOMACH WITH SPECIAL REFERENCE TO EARLY CARCINOGENESIS. *(E.)* Kanahara, H. (Niigata Univ. Sch. Med., Japan). *Acta Med Biol (Niigata)* 21(3/4): 133-168, 1974.

Atypical proliferations of the epithelium produced at the margin of chronic ulcers in the glandular stomach of animals were observed in an effort to follow (chronologically) changes leading to possible malignant transformation. Experimental ulcers were induced by lantan-gel injection and electrocoagulation in mastomys; Donryu rats of both sexes were used as controls. In another experiment, the carcinogenic effects of N-methyl-N'-nitro-N-nitrosoguanidine on epithelial proliferations of experimental chronic gastric ulcers in mastomys and Wistar rats were examined. Ulcers at the site of the lantan-gel treatment and at the site of gastrotomy showed a higher incidence of chronic lesions than those at the site of electrocoagulation. On the basis of histologic analysis of chronic ulceration, fibroblastic fibrosis associated with particles of lantan-gel and suture material in the ulcer base was considered to be the main interference to ulcer healing, thus resulting in chronic ulceration. The types of epithelial proliferation associated with chronic ulceration can be histologically divided, in order of chronological changes, into regeneration (simple epithelial proliferation), glandular proliferation, adenomatous proliferation, atypical epithelial proliferation, and malignant proliferation. The longer the experimental period, the higher the incidence of atypical epithelial proliferation. Lantan-gel ulcers followed by N-methyl-N'-nitro-N-nitrosoguanidine gave a high incidence of chronic ulceration (15/25; 60%). Various grades of atypical epithelial proliferation at the ulcer margin and base were produced by N-methyl-N'-nitro-N-nitrosoguanidine administration to Wistar rats bearing lantan-gel ulcers; four cases of these produced adenocarcinomas. The results suggest that marginal epithelial proliferation of an experimental chronic gastric ulcer indicates the possibility of transformation to atypical and even malignant changes.

6375 CUTANEOUS MANIFESTATION OF HODGKIN'S DISEASE. SYSTEMATIC STUDY OF 94 PATIENTS WITH HODGKIN'S DISEASE. *(Fr.)* Dugois, P. (No affiliation), P. Amblard, J. Martel, R. Schaerer and J. P. Fillon. *Bull Soc Fr Dermatol Syphiligr* 80(3):309-313, 1973.

A sample of 94 patients with Hodgkin's disease, studied for the cutaneous manifestations of the disease, included 56 (59%) who had a cutaneous lesion at some time during disease development. There were four cases of specific lesions, two representing diffuse Hodgkin's disease, one proceding from the proximity of adenopathic involvement, and one of primary cutaneous Hodgkin's disease. In the last case, the rapidly fatal development correlated with the immunological test results of extensively retarded hypersensitivity. Non-specific cutaneous manifestations included pruritis (30 cases), shingles (12 cases), ichthyosis (four cases), xeroderma (nine cases), and spinulosism (four cases). Immunological depression was demonstrated for the patients with shingles, but not in the other instances of cutaneous manifestations. Seven patients with no cutaneous lesions showed minimal immunological depression. It is suggested that cutaneous lesions give some indication of the ability of a patient to oppose malignant proliferation.

6376 PRIMARY THYMIC HODGKIN'S DISEASE: A CLINICOPATHOLOGIC STUDY OF 27 CASES. *(E.)* Fazekas, J. (Univ. S. California Sch. Med., San Francisco), C. Greenberg and O. N. Ramba. *Radiol Clin Biol* 43(5):456-464, 1974.

Twenty-seven patients with primary thymic Hodgkin's disease were studied in order to determine if the thymus is involved in the spread of Hodgkin's disease, and is similar to lymphatic Hodgkin's disease in histological appearance and clinical course and treatment. Thirteen male and 14 female patients were examined. Cervical lymph node enlargement, respiratory complaints and chest pain were seen in 37%, 33%, and 26% of the patients respectively. Seventeen patients had supraclavicular lymphadenopathy when diagnosed. The histologic pattern observed was similar to that seen in patients with nodular sclerosing Hodgkin's disease. A 40% cure rate was achieved in these patients, and was observed to be independent of disease stage or radiation dose. Initial irradiation of both supraclavicular fossae (regardless of palpable adenopathy) resulted in higher cure rates. A therapeutic regimen consisting of tumor doses of 3,500-4,000 rad is recommended, since higher doses contribute to morbidity and even to mortality. Although the thymus gland cannot be considered an integral part of the lymphatic system in respect to tumor metastasis, it appears to be a frequent if not the sole primary site of nodular sclerosing Hodgkin's disease.

6377 AN INVESTIGATION INTO THE ENZYME HISTOCHEMISTRY OF ADENOCARCINOMAS OF THE HUMAN LARGE INTESTINE AND OF THE TRANSITIONAL EPITHELIUM IMMEDIATELY ADJACENT TO THEM. *(E.)* Marsden, J. R. (Dep. Pathol., Univ. Nottingham, England) and I. M. P. Dawson. *Gut* 15(10):783-787, 1974.

Histochemical enzymatic studies were performed on 30 freshly resected large bowel carcinoma, 30 samples of normal colonic epithelium, and six samples of histologically normal epithelium (transitional epithelium) immediately adjacent to a carcinoma. Five enzymes were studied: nicotine adenine dinucleotide tetrazolium reductase (NADH-TR), glucose-6-phosphate dehydrogenase, succinate dehydrogenase, monamine oxidase, and acid phosphatase. Quantitative and qualitative differences in enzyme activity were observed between normal, transitional, and carcinomatous mucosa as follows: monamine oxidase activity was moderate in normal mucosa, high in transitional mucosa and low in carcinoma. Succinate dehydrogenase activity was high in transitional mucosa and low or moderate in normal and carcinomatous mucosa. Glucose-6-phosphate dehydrogenase activity showed a gradation from low in normal mucosa to high in carcinoma while acid phosphatase showed the reverse of this pattern. NADH-TR activity was low or moderate in normal and transitional mucosa and high in carcinoma. It is emphasized that qualitative changes were limited to small differences in localization of the enzyme between normal and carcinomatous mucosa. All the enzymes, except NADH-TR, showed differences of staining intensity in transitional mucosa from the pattern obtained for normal or cancerous mucosa. This could be a manifestation of developing cancer cell metabolism or of normal cellular adaption to a changed environment. The findings of this study may have clinical significance in the assessment of precancerous lesions.

6378 BLUE HISTIOCYTES AND GAUCHER CELLS IN THE SPLEEN AND LYMPH NODES IN CHRONIC MYELOGENOUS LEUKEMIA. *(Fr.)* Hopfner, C. (Hosp. Cent. Univ. Reims, France), G. Potron, J.-J. Adnet, T. Caulet and J. Boy. *Nouv Rev Fr Hematol* 14(5):607-620, 1974.

A case of chronic myelogenous leukemia in a 73-yr-old woman was studied. Histochemical and ultrastructural studies of spleen and peripheral lymph node biopsies revealed the presence of blue histiocytes, Gaucher cells, and transitional cells. The type of stored material appeared to be morphologically variable for different macrophages, but the cells were so intimately mixed that histochemical analysis of the cytoplasmic content was extremely difficult. The blue histiocytes had the characteristics of ceroids, rich in poly-unsaturated fatty acids. The lipid store of the blue histiocytes and transitional cells was complex. The Gaucher cells showed the morphological appearance and the histochemical color changes of the cells of Gaucher's disease, but the ultrastructural study showed a different storage material. There were no tubular formations of the glucocerebrosides of Gaucher's disease, but rather linear lamellar structures. The evidence of many instances of phagocytosis and of several stages of storage cells favors the hypothesis of a functional connection between the storage cells and the excessive cytolysis of chronic myeloid leukemia and other hematological diseases.

6379 RADIOMORPHOLOGICAL CHARACTERISTICS OF THE DEVELOPMENT AND EVOLUTION OF PRIMARY CARCINOMA OF THE LUNG. *(E.)* Schweiger, O. ("Koranyi" Natl. Inst. Tuberc. Pulmonol., Budapest, Hungary), M. Barabas and A. Tamas. *Scand J Resp Dis Suppl* 89:65-78, 1974.

The development and evolution of the radiomorphological features of primary bronchus carcinoma was studied in 128 patients with isolated homogenous coin lesions and 56 patients with primary bronchus carcinoma with infiltrative-like opacity. The development of the carcinoma followed three different patterns: after a previous negative image, an isolated, homogenous, sharp-edged opacity appears and continues to grow; a spindle-formed opacity precedes the appearance of a small, regular, round nodule which grows gradually; or pneumonia (or bronchopneumonia) appears first in the region which will later contain the carcinoma, the latter growing until it becomes visible on the radiograph. The first form is by far the most common, with the latter two being about equally common. The radiological characteristics of the evolution of the primary bronchus carcinoma showed two types: simple growth of the coin lesion; or appearance of the radiological characteristics of primary bronchus carcinoma. In the latter case, the tumor may be lobulated, there may be cobweb-like linear shadows, a cavity or excavation may appear inside the coin lesion, a thick stripe may pass from the coin lesion to the pleura or diaphragm, or there may be consecutive lesions. The most common forms are the coin lesion and the lobulated tumor. Better utilization of all the possible methods of radiological examination will lead to the definition of many additional aspects of this disease.

6380 CUTANEOUS XANTHOMATOSIS AND MULTIPLE MYELOMA: ANALYSIS AND REVIEW. *(E.)* Krain, L. S. (No affiliation). *Cutis* 14(3):423-427, 1974.

Fifty-seven cases of cutaneous xanthomatosis are reviewed and analysed for associated multiple myeloma and myeloma-like diseases. Twenty cases of cutaneous xanthomatosis with multiple myeloma are analysed with regard to such factors as age, race, sex, serum abnormalities and consideration of familial cutaneous xanthomatosis. It is suggested that IgG may act as an autoantibody, preventing arteriosclerosis formation; IgA may combine with beta lipoproteins, preventing their clearance from the system, which may lead to hyperlipidemia and hypercholesterolemia with resultant arteriosclerosis and xanthoma formation. Therefore it is advised that patients over age forty with cutaneous xanthomatosis should be routinely examined for associated multiple myeloma. (27 references)

6381 EFFECTS OF EACA, AMCHA AND GUANETHIDINE ON METASTASES INDUCED BY INTRAVENOUSLY INJECTED TUMOUR CELLS. *(E.)* Boeryd, B. (Dep. Pathol., Univ. Göteborg, Sweden), B. Hagmar, G. Johnsson and W. Ryd. *Pathol Eur* 9(2):119-123, 1974.

The influence of an antifibrinolytic drug, aminomethyl-cyclohexane carboxylic acid (AMCHA), on metastasis formation was compared with that of a vasoactive drug, guanethidine, and with a drug with both these effects, ε-aminocaproic acid (EACA). Two syngeneic tumor host systems were used: a methylcholanthrene-induced sarcoma (MCG1-SS) in CBA mice and myeloma B16 in C57BL/6J mice. The drugs were administered i.p. 30 min before i.v. injection of 10^5 B16 cells or 5×10^4 MCG1-SS cells. EACA (60 mg) increased lung metastases formation from tumor cells in both systems. AMCHA (12 mg) and guanethidine (0.6 mg) increased only B16 lung metastases. That all three compounds increased the yield of B16 pulmonary metastases, while only EACA affected MCG1-SS metastases, might be due to the thromboplastic properties of B16. AMCHA, in addition to its antifibrinolytic activity, also affects the sympathetic nervous system and thereby has hemodynamic effects. Possibly, increased formation of pulmonary metastases of MCG1-SS occurs only if the effects on circulation and fibrinolysis are combined. The effect of AMCHA on the B16 metastases can be explained by its antifibrinolytic activity and/or its guanethidine-like effects. These results demonstrate the importance of using more than one tumor-host system when testing the effect of a drug on the development of metastases. The data also indicate that EACA's hemodynamic effects are important co-factors affecting metastasis.

6382 OBSERVATIONS ON THE EFFECT OF NEURAMINIDASE ON THE DISTRIBUTION OF INTRAVENOUSLY INJECTED WALKER TUMOR CELLS IN RATS. *(E.)* Weiss, L. (Dep. Exp. Pathol., Roswell Park Mem. Inst., Buffalo, N.Y.), B. Fisher and E. R. Fisher. *Cancer* 34(3):680-683, 1974.

Investigations were undertaken to explain the events involved with tumor cell attachment to vascular epithelium, an initial step in metastasis. Walker 256 tumor cells were treated with neuraminidase; treated and control Walker 256 cells were labeled with ^{51}Cr, injected into femoral or portal veins of rats and γ-counts of organs were made five min and one hr later. Little or no difference in the organ cell counts was seen after injection of either neuraminidase-treated or untreated cells. Calculations based on electrophoretic mobility tests and previously published data gave total interaction energies. These data indicate that it is unlikely that arrest of tumor cells occurs due to simple collisions with vascular endothelium or that this process would be directly affected by the 30% reduction of surface negativity produced by neuraminidase. In considering the possibility that thrombogenic factors are involved with attachment of tumor cells to vessel walls, it is calculated that platelets would collide 1% with untreated cells and 20% with treated cells. Neuraminidase-susceptible, cell surface sites did not influence hematogenous metastasis.

6383 THE CONFRONTATION OF NORMAL TISSUE AND MALIGNANT CELLS *IN VITRO:* HUMNA DECIDUA GRAVIDITATIS AND HELA CELLS. A MODEL FOR STUDIES ON TUMOR INVASION. *(E.)* Schleich, A. (German Cancer Res. Cent., Heidelberg, West Germany), M. Frick and A. Mayer. *Z Krebsforsch* 82(3):247-255, 1974.

The non-malignant tissue, human decidua graviditatis, was exposed *in vitro* to malignant HeLa cells (a strain of an adenocarcinoma of the cervix) to study the invasion and infiltration by malignant aggressor cells into non-malignant receptor tissue. Both the normal tissue and the cancer cells were cultivated separately in media consisting of 79% basal medium Eagle, 1% glutamine, 20% fetal bovine serum, 200 U penicillin, 100 U streptomycin and 100 μg kanamycin/ml. The normal tissue (decidua parietalis), behaving like corresponding, differentiated tissue in the body, tried to repair lesions, but did not proliferate. The HeLa cells formed miniature tumors which are histologically carcinoma. When fragments of the HeLa cells were applied to the normal tissue, the cancer cells spread on the surface of the decidua and penetrated into the grandular lumina. The zones of interaction between non-malignant and malignant cells were well defined and unaffected by inflammatory reactions, thus suitable for studies on the release of lytic enzymes by tumor cells. This system thus represents an *in vitro* model of the invasion by malignant cells into non-malignant receptor tissue.

6384 ROLE OF SOMATIC CELL FUSION IN THE PROGRESSION OF MALIGNANCY. *(E.)* Goldenberg, D. M. (Univ. Kentucky Med. Cent., Lexington) and R. A. Pavia. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:290-297, 1974.

Karyotype studies of a hybrid tumor cell line, which originated through *in vivo* fusion of human lymphoid tumor cells with hamster cells by implantation of the tumor cells into hamster cheek pouches were described. The resultant highly malignant transplantable hamster tumor was shown to contain both human and hamster gene-determined characteristics. For these studies the tumor was converted first to an ascites form, then propagated *in vitro* for 2-17 months, the resulting cell populations containing no hamster fibroblasts, an absence of diploid cells and numerous chromosomal anomalies. Gel electrophoresis showed lactic dehydrogenase isozyme banding from these cells to be typical of hamster whereas glucose-6-phosphate dehydrogenase, similarly studied, showed band patterns characteristic of hamster fibroblasts and HeLa cells. Karyotypes of hamster cheek fibroblasts and mouse melanoma cells (Cloudman S91, melanotic) were performed by fusing these cell types *in vitro*, via lysolecithin, for six weeks. A population of hyperdiploid cells was obtained with karyotypes resembling hamster more than melanoma but chromosome band patterns showed some sharing of both genomes, and these hybrid cells were highly malignant to hamsters. The results support the proposed role for somatic cell fusion of malignant and normal host cells as a natural phenomenon of neoplasia.

6385 RETICULOHISTIOCYTOMA OF ADULT'S BACK: LIMITED CUTANEOUS RETICULOSIS OF SLOW EVOLUTION. *(Fr.)* Laugier, P. (Cantonal Hosp., Geneva, Switzerland), N. Hunziker and L. Olmos. *Dermatologica* 149(6):350-358, 1974.

A case of reticulohistiocytoma of the back is reported in a 56-year-old man who seemed in good physical condition, suffered no discomfort, and worked normally, but lost 13 kg in a six-month period. The first tumor appeared about ten yr earlier as a flat red spot in the middle of the patient's back. This hard, painless lesion slowly increased in size; turning purple with raised edges. A second tumor, smaller but similar, appeared a few yr later. Histological examination of biopsy tissue showed dense infiltration, almost exclusively composed of histiocytes with enormous, irregular nuclei. The ultrastructural study corroborated the monstrous size and contorted appearance of the nucleus as well as the lack of nuclear membrane, thereby confirming the dysplastic and malignant character of the cells. The patient refused treatment and returned to work.

6386 ULTRASTRUCTURE OF ABRIKOSSOFF'S GRANULAR CELL TUMOR. *(Fr.)* Bourlond, A. (Dermatol. Clin. Univ. Louvain, Belgium) and H. Degreef. *Arch Belg Dermatol* 30(3):159-166, 1974.

A 21-year-old man with multiple lesions of the upper limbs was studied. The lesions were small, painless, s.c. tumors (up to 2 cm in diameter) of firm consistency, either pinkish-red or of normal skin color. Histological examination of biopsy specimens revealed the presence of Abrikossoff's tumors. The results of the ultrastructural analysis led to the conclusion that the granular cells proliferate and envelop the nerves prior to causing various alterations. A review of the literature seems to indicate that mesenchymal and Schwann cells might be able to produce Abrikossoff's granular cell tumors.

6387 THE EARLY STAGE OF A NEOPLASTIC GROWTH IN THE TESTIS OF A PATIENT WITH MIXED GONADAL DYSGENESIS - ULTRASTRUCTURAL AND HISTOLOGICAL OBSERVATION. *(E.)* Belicza, M. (Fac. Med., Zagreb, Yugoslavia), P. Drobnjak, V. Grizelj and I. Puharic. *Acta Med Iugosl* 28(4):355-368, 1974.

Histological and electron microscope examinations were made of specimens of gonads from a woman (aged 55 yr) with mixed gonadal dysgenesis. Two different types of tissues were seen in the testis in early stages of neoplastic growth; little clumps of anaplastic cells (epithelial or endothelial in origin) and an early stage of an interstitial Leydig cell tumor with a "lipoid-cell tumor-like" or "adrenal rest-like" appearance. A small number of secretory granules (dense bodies) in the Leydig cells examined could be correlated with the small production of androgen steroids by the patient, but the findings were speculative and inconclusive.

6388 LEYDIG CELL TUMORS. FINE STRUCTURAL STUDY. *(Sp.)* Nistal, M. ("La Paz" Polyclin. Social Security, Madrid, Spain), F. Rubio Lopez and J. Figols. *Arch Esp Urol* 27(3):301-318, 1974.

6389 HEPATIC CARCINOMA. ONE-CASE PRESENTATION. *(Sp.)* Fundora Mayor, I. (Dept. Pathoanat., "Cmte. M. Fajardo" Hosp., Cuba) and A. Paramio Ruibal. *Rev Cubana Cir* 13(6):691-697, 1974.

6390 OBSERVATIONS OF NUCLEAR INCLUSIONS IN MIXED TUMORS OF THE PAROTID GLAND. *(It.)* Motta, G. (Clin. Otorhinolaryngol., Univ. Cagliari, Italy) and A. Riva. *Arch Ital Anat Istol Patol* 45(1-3):71-101, 1974.

6391 ASSOCIATION OF CANCER OF THE VULVA AND CANCER OF THE UTERINE CERVIX. ELECTRON MICROSCOPIC STUDY OF THE TWO TUMORS. *(Sp.)* Clavero Nuñez, J. A. ("Francisco Franco" Provincial Health Center, Madrid, Spain), F. Sánchez-Garrido and I. Herrera. *Acta Ginecol* 25(10):791-802, 1974.

6392 COIN LESIONS OF THE LUNGS AS AN INITIAL STAGE OF BRONCHIAL CARCINOMA. *(Cro.)* Mesic, J. (Clin. Hosp., Ljubljana, Yugoslavia). *Plucne Bolesti Tuberk* 26(Suppl. 2):320-323, 1974.

6393 DYSKERATOTIC SQUAMOUS-CELL EPITHELIOMA OF THE NAIL-BED. ULTRASTRUCTURAL STUDY. *(Fr.)* Temime, P. (Marseille, France), J. J. Bonerandi, Y. Privat, J. C. Laugier and A. Martinetti. *Bull Dermatol* 81(3):341-347, 1974.

6394 DIFFUSE HETEROTOPIC MULTIPLE CYSTS AND CARCINOMA OF THE STOMACH. *(Jpn.)* Iwanga, T. (Cent. Adult Dis., Osaka, Japan). *Gan No Rinsho* 19(10): 971-979, 1973.

6395 ASSOCIATION BETWEEN METASTASES IN BREAST CANCER AND THE PRESENCE OF CARCINOEMBRYONIC ANTIGEN. *(E.)* Opler, S. R. (Stanford Univ., Calif.). *Fed Proc* 33(3):638, 1974.

6396 REGULATION OF MEMBRANE STRUCTURE IN TRANSFORMED CELLS. *(E.)* Furcht, L. T. (Univ. Minnesota, Minneapolis), R. E. Barnett, N. D. Goldberg and R. E. Scott. *Fed Proc* 33(3):629, 1974.

6397 HEART TUMORS IN MICE OF THE T.M. STRAIN MAINTAINED ON FAT ENRICHED DIETS. *(E.)* Szepsenwol, J. (Univ. Puerto Rico Sch. Med., San Juan) and N. V. Boschetti. *Fed Proc* 33(3):364, 1974.

6398 THE ASSOCIATION OF NEOPLASMS OF THE COLON AND RECTUM. WITH PRIMARY MALIGNANCIES OF OTHER SITES. *(E.)* Schoenberg, B. S. (Mayo Grad. Sch. Med., Rochester, Minn.) and B. W. Christine. *Am J Proctol* 25(4):41-58, 1974.

6399 NEOVASCULARIZATION INDUCED BY INTRAOCULAR XENOGRAFTS OF NORMAL, PRENEOPLASTIC AND NEOPLASTIC MOUSE MAMMARY TISSUES. *(E.)* Gimbrone, M. A., Jr. (Natl. Inst. Health, Bethesda, Md.) and P. M. Gullino. *Fed Proc* 33(3):596, 1974.

6400 BIOLOGIC BEHAVIOR OF CONGENITAL MELANOMAS IN SINCLAIR MINIATURE SWINE. *(E.)* Carey, K. D. (Sch. Vet. Med., Univ. Missouri, Columbia) and P. J. Manning. *Fed Proc* 33(3):596, 1974.

6401 INTERMITTENT SPHERING PROPERTY OF NEOPLASTICALLY TRANSFORMED CELLS OBSERVED BY TIME LAPSE CINEMATOGRAPHY. *(E.)* Paranjpe, M. (Natl. Inst. Health, Bethesda, Md.) and C. W. Boone. *Fed Proc* 33(3):604, 1974.

6402 EFFECT OF SEX-LINKED ABROGATION OF THE GRAFT-VERSUS-HOST-REACTION (GVHR) ON PATHOLOGIC CHANGES, INCLUDING TUMOR INDUCTION. *(E.)* Cornelius, E. A. (Yale Univ. Sch. Med., New Haven, Conn.). *Fed Proc* 33(3):615, 1974.

6403 A CASE OF ACUTE LEUKEMIA IN A GIRL WITH FANCONI'S SYNDROME: LITERATURE REVIEW. *(Fr.)* Levy, J. M. (Univ. Hosp. Cent., Strasbourg, France), C. Stoll and R. Korn. *Nouv Rev Fr Hematol* 14(6):713-720, 1974.

6404 THE COEXISTENCE OF MYELOMA AND GRANULOCYTIC LEUKEMIA IN THE ABSENCE OF TREATMENT: STUDY OF FOUR CASES. *(Fr.)* Tursz, T. (St. Louis Hosp., Paris, France), G. Flandrin, J. C. Brouet and M. Seligmann. *Nouv Rev Fr Hematol* 14(6):693-704, 1974.

6405 BILIARY CARCINOMAS. *(Ger.)* Moschinski, D. (Surg. Clin., Univ. Dusseldorf, Germany), R. Sailer and G. Jacobs. *Bruns Beitr Klin Chir* 221(8):592-598, 1974.

6406 ESOPHAGEAL PAPILLOMATOSIS AND ACANTHOSIS NIGRICANS. *(Fr.)* Heully, F. (Brabois Hosp., Vandoeuvre, Belgium), P. Gaucher, M. Weber, R. Jeanpierre, J. Laurent, B. Kremer and J. Beurey. *Ann Gastroenterol Hepatol* 10(6):487-490, 1974.

6407 CURRENT NOSOLOGIC CONCEPTS ABOUT MALIGNANT NON-HODGKIN LYMPHOMAS. *(Fr.)* Gerard-Marchant, R. (Gustave Roussy Inst., Villejuif, France). *Ann Anat Pathol (Paris)* 19(2):149-162, 1974.

6408 ULTRASTRUCTURAL STUDY OF SIX PHEOCHROMOCYTOMAS: PRESENCE OF INTRACELLULAR CANALICULI. *(Fr.)* Oliva, H. (Jimenez Diaz Fdn., Madrid, Spain) and G. Usera. *Ann Anat Pathol (Paris)* 19(3):287-298, 1974.

6409 DUBREUILH'S CIRCUMSCRIBED PRECANCEROUS MELANOSIS. *(Ger.)* Tilgen, W. (Univ. Clin. Dermatol., Heidelberg, West Germany) and U. W. Schnyder. *Virchows Arch [Pathol Anat]* 363(2):163-174, 1974.

6410 TRANSFORMATION OF APLASTIC ANEMIA IN ACUTE LEUKEMIA. *(Sp.)* Santiago Aranda, I. (Service Hematol., Gen. Hosp., Mexico City, Mexico), R. Gonzalez Constandce and M. Gutierrez Romero. *Sangre (Barc)* 19(4):450-455, 1974.

6411 BLOOD CIRCULATING CELLS WITH HIGH DNA CONTENT IN TWO PATIENTS AFFECTED BY MALIGNANT LYMPHOMA. *(It.)* Chilosi, M. (Inst. Pathoanat. Histopathol., Munic. Hosp., Udine, Italy) and M. Nigro. *Pathologica* 66(957-8):287-293, 1974.

6412 ON MALIGNANT MELANOMA. *(Ger.)* Stein, G. (Dept. Radiol., Univ. Clin. Dermatol., Bonn-Venusberg, Germany). *Radiobiol Radiother (Berl)* 15(4):475-479, 1974.

6413 EOSINOPHILIA IN ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOSARCOMA: INDICATOR OF IMMUNE RESPONSE? *(Ger.)* Kaufmann, U. (Univ. Clin. Pediatr., Giessen, West Germany) and F. Lampert. *Klin Paediatr* 186:492-499, 1974.

6414 A CASE OF TRANSFORMATION OF POLYCYTHAEMIA VERA IN BLAST LEUKOSIS. *(Bul.)* Atanassov, K. (Dept. Int. Med. Ther., Fac. Med., Plovdiv, Bulgaria) and A. Kolistarkov. *Vutr Boles* 13(3):117-120, 1974.

6415 A NEW CASE OF MYOID TUMOUR OF THE STOMACH. ENDOSCOPIC, ARTERIOGRAPHIC AND PATHOLOGICAL STUDY. *(Fr.)* Gillet, M. (Clin. Surg., Univ. Hosp., Besancon, France), J. Kahn, G. Camelot, J. Elbaz, C. Monange, G. Pageaut and F. Weill. *Chirurgie* 100(12):825-829, 1974.

6416 NEUROBLASTOMA IN CHILDHOOD AS A BIOLOGICAL PROBLEM. *(Cz.)* Stasek, V. (Oncol. Clin. Labor., Charles Univ., Prague, Czechoslovakia), J. Jakoubkova, J. Koutecky and J. Bubenik. *Vnitr Lek* 20(6):609-612, 1974.

See also:

* (Rev): 6005, 6006, 6040, 6041, 6043, 6044, 6059, 6062, 6070, 6071
* (Chem): 6092, 6093, 6104, 6107, 6127, 6131, 6135, 6136, 6144, 6153, 6154
* (Phys): 6176
* (Immun): 6306, 6328
* (Epid-Biom): 6422, 6423, 6431, 6444

6417 CANCER STATISTICS, 1974--WORLDWIDE EPIDEMIOLOGY. *(E.)* Silverberg, E. (Am. Cancer Soc., New York, N.Y.) and A. I. Holleb. *CA* 24(1): 2-21, 1974.

Epidemiologic data are presented for cancer of the following sites: stomach, colon, rectum, lung, breast, uterine cervix, prostate and bladder. Data are also presented for leukemia. Stomach cancer mortality rates are high in Chile, Japan, Austria, Iceland, Central Europe, Finland, and U.S.S.R., but low in the U.S., Australia, New Zealand, Canada, and Israel. A diet rich in starches and low in fresh fruits and vegetables seems to be related etiologically. Mortality rates from colon and rectal cancer are high in North America, Australia, New Zealand, and Eastern Europe, but low in Central America and the Caribbean, Sub-Saharan Africa, the Eastern Mediterranean, Southwest Asia and Japan. There is an increased risk of colon and rectal cancer in patients with a history of ulcerative colitis, diverticulitis, appendicitis, polyps, or familial polyposis. Again, diet has been implicated etiologically. Lung cancer rates are high wherever death-registration systems are used and low in most of Africa and Asia. Multiple factor relationships have been observed in cigarette smokers exposed also to either asbestos or radon. Death rates from breast cancer are high in many countries, but low in the U.S.S.R., Japan, parts of the Far East, Africa and the Caribbean. The identifiable risk groups are indicated. Morality rates for cancer of the uterine cervix are high in Latin America and in parts of Africa and Asia, but are low in Europe, North America, Australia and New Zealand. Cervical cancer is related to sexual activity and is uncommon in communities where cleanliness is stressed. Prostatic cancer death rates are high in Canada, the U.S., France, Belgium, Netherlands, Switzerland, Scandinavia, Australia, and New Zealand, but low in most of Asia, Africa, and Latin America. Some relationship may exist between endocrine function and prostatic cancer. Mortality rates from bladder cancer are high in parts of South America, England, Scotland, and the U.S.; death rates are low in Asia, several Caribbean countries, Ireland, Norway, and Sweden. Persons at risk include cigarette smokers, patients with a history of chronic bladder infection or kidney stones, and aniline dye workers. Leukemia death rates show slight variations throughout the world, but they are usually higher in Jews than in Catholics or Protestants. Leukemia can be caused by excessive exposure to radiation; its relationship to viruses remains unclear. Estimates of cancer incidence are based on rates derived from the National Cancer Institue's Third National Cancer Survey.

6418 RELATION OF PLACE OF BIRTH AND MIGRATION IN CANCER MORTALITY IN THE U.S.--A STUDY OF OHIO RESIDENTS (1959-1967). *(E.)* Mancuso, T. F. (Dep. Occup. Health, Univ. Pittsburgh, Pa.) and T. D. Sterling. *J Chronic Dis* 27(9/10):459-474, 1974.

Mortality studies of Ohio residents (1959-1967) born in the United States were conducted to explore demographic, nativity, migration, and industrial variables. Thirteen individual cancer sites and combinations of malignancies were considered and the age adjusted rates computed by sex and race. The age adjusted rate for all cancers (ages 35-69) for the nonwhite males born in the South were 363.67 versus 180.43 for all those born in Ohio. The same ratio among the white males born in the South was 225.73 and for those born in Ohio, 197.14. For cancer of the lung and other specific cancer sites, the nonwhite males showed a higher rate for those born in the South compared to those born in Ohio. The excess for the white males was most notable for cancer of the lung. Statistical evidence is presented for the conclusion that the observed differences in cancer mortality among the nonwhites are not due to race. The importance of endemic factors associated with place of birth in the United States and the influence of the early years of life, particularly in the South in the development of social and biological imprints, and following migration the subsequent exposure to industrial environments was considered. This study demonstrates the marked differences in cancer mortality which become manifest for the nonwhite and native born white when resident male and female deaths of a state are subclassified by region of birth and state of residence, which would not otherwise be apparent in the comparisons by broad classifications as currently coded in vital records.

6419 FACTOR ANALYSIS OF INTERNATIONAL CANCER MORTALITY DATA AND *PER CAPITA* FOOD CONSUMPTION. *(E.)* Howell, M. A. (Natl. Cancer Inst., Bethesda, Md.). *Br J Cancer* 29(4):328-336, 1974.

Relationships among cancer sites in males were analyzed based on international mortality data for 1964-1965 in 41 countries and correlated to *per capita* food consumption figures available in 36 countries. The westernization factor and two other factors accounted for the major part of the variance in international cancer death rates. The second factor represented stomach cancer with which cancer of the larynx was associated, perhaps because of correlations of the death rates for these sites with other variables not included in the analysis. The third factor was a buccal-esophageal cancer factor which was interpreted as reflecting common etiological agents in the development of carcinomas of these sites. A number of food correlates of the westernization factor were found, one of which (cattle meat) was interpreted as having significance in the development of colorectal cancer in view of similar findings from a case control study. The stomach cancer factor was associated with starch consumption, specifically potatoes, which also is supported by case control results. Cancer of the larynx was associated with the consumption of vegetables, fruit and wheat. No explanation of this finding was found. Death rates from buccal-esophageal cancers were found not to be associated with dietary factors. Factor analysis provides a meaningful summary of correlations and clarifies the observed statistical associations. It could prove a useful epidemiological tool.

6420 A REVIEW OF SOME TUMORS OF INTEREST FOR DEMOGRAPHIC STUDY IN HAWAII. *(E.)* Rhoads, G. G. (Sch. Public Health, Univ. Hawaii, Honolulu), G. A. Glober and G. N. Stemmermann. *Hawaii Med J* 33(8):283-291, 1974.

Tumor incidence rates among the major ethnic groups in Hawaii, and between a given immigrant group and its country of origin, were compared. Tumors were identified which have an incidence substantially different from those in the three oriental countries of origin (China, Japan, Philippines) and in the U.S., or have substantially different incidence among the different ethnic groups. These tumors are cancer of the nasopharynx, stomach, prostate, large intestine, liver, breast, uterine corpus, ovary, bladder and thyroid. Because of its multiracial character, Hawaii provides a unique opportunity to study important environmental determinants of common cancer. Two major problems that will be encountered in such a study are disentangling multiple variables and the time-lag between environmental influences and actual appearance of chronic disease.

6421 INVESTIGATION OF THE CONTAMINATION OF HUNGARIAN CEREALS BY CARCINOGENIC POLYAROMATIC HYDROCARBONS.

(Hun.) Soos, K. (Natl. Inst. Food Nutr. Sci., Budapest, Hungary). *Elelmiszervizsgalati Kozl* 19(6):299-307, 1973.

Sixteen wheat grain, 11 barley grain, and six oat grain samples from different areas of Hungary were analyzed for carcinogenic polyaromatic hydrocarbons by UV-spectrophotometric method. Wheat grain samples contained 1.25 μg/kg pyrene, 0.87 μg/kg fluoroanthene, 0.21 μg/kg 1,2-benzoanthracene, 0.16 μg/kg chrysene and 1,2-benzopyrene each, 0.23 μg/kg 3,4-benzo(a)-pyrene, 0.02 μg/kg 1,2,5,6-dibenzoanthracene, and 1,12-benzoperylene. The corresponding levels in barley grains were 1,67; 2.05; 0.5; 0.34; 0.82; 0.05, and 0.16 μg/kg. Barley grains also contained traces of perylene and antanthrene, as well as 0.03 μg/kg coronene. Oat grains contained 1.90 μg/kg pyrene, 2.29 μg/kg fluoroanthene, 0.53 μg/kg 1,2-benzoanthracene, 0.19 μg/kg chrysene, 0.22 μg/kg 1,2-benzopyrene, 0.87 μg/kg 3,4-benzo(a)pyrene, 0.01 μg/kg coronene. The contamination levels were about 2-4 times higher in samples from fields near highways and industrial plants than they were in samples from areas with low air pollution level, which indicates that the polyaromatic hydrocarbons originate mainly from industrial and vehicle exhaust emissions. There was no difference in the contamination levels of non-dried and artificially dried grains. The processing of wheat to flour and bakery products was found not to cause any reduction in the carcinogen polyaromatic hydrocarbon contents. The possible accumulation of carcinogenic hydrocarbons present in barley and oats in animals, and their possible passage into humans will require further investigations.

6422 ASBESTOSIS IN WORKERS OF AN INDUSTRIAL PLANT IN LOWER SILESIA.

(Pol.) Kaniowski, T. (Med. Acad., Warsaw, Poland), D. Randowa and J. Wozniak. *Pol Przegl Radiol* 38(5):575-583, 1974.

Between 1968 and 1971, radiologic studies and pulmonary function tests were performed on 108 of 400 workers at a Lower Silesian industrial plant processing asbestos. Diagnosis of asbestosis was made solely on the basis of radiologic changes in 20 workers (12 women and eight men, aged 39-65 yr). All had been exposed to asbestos dust for 10 to 24 years. The most common radiologic changes were intraparenchymal: 15 were quite advanced and five were in the initial stages. In eight cases they were accompanied by emphysema. All but one had symptoms of respiratory impairment which was aggravated on exertion and was accompanied by hypoxia. In eight the insufficiency was of the restrictive, in five of the obstructive and in six of the mixed type. In seven the oxygen saturation of arterial blood decreased 88-93% after exertion; in one case there was an increase in P_{CO_2} pressure to 50 mmHg and compensated respiratory acidosis after exertion. None of the subjects had decompensated acidosis. None had signs of lung cancer, and no pleural tumors were found. In three cases asbestosis was accompanied by histological evidence of tuberculosis.

6423 CHEMICAL LEUKEMOGENESIS IN MAN.

(E.) Forni, A. (L. Devoto Clin., Univ. Milan, Italy) and E. C. Vigliani. *Ser Haematol* 7(2):211-233, 1974.

Examples of benzene leukemia are reported, including 81 cases of benzene hemopathy in the provinces of Milan (1960-1966) and Pavia (1960-1964). The cases occurred in shoe factories and rotogravure plants where benzene was used as a solvent for glues and inks, resp. Forty-nine patients with anemia are alive, while 32 are dead from anemia (14), acute leukemia (17), and erythroleukemia (1). In the 1960's the risk of acute leukemia in the exposed Italian workers was at least 20 times higher than that in the general population. In the Paris region (1950-1963), 44 cases of leukemia were identified in subjects with chronic benzene exposure; 23 had acute leukemia, 13 chronic myeloid leukemia, and 8 chronic lymphocytic leukemia. The Italian series differed from the Paris series in that acute leukemia occurred at the same time as cases of aplastic leukemia only in factories where benzene concentrations were well above the threshold level. Data from Lyon hospitals showed a significantly increased incidence of acute leukemia, chronic lymphocytic leukemia, and aplastic anemia in workers chronically exposed to benzene or its homologues. These epidemiological data favor a leukemogenic action of benzene in man, either directly or through aplastic changes in bone marrow. Animal studies indicate that benzene can severely disturb DNA synthesis in hemopoietic cells and induce chromosome aberrations in bone-marrow cells. Other suspected leukemogens are chloramphenicol, phenylbutazone, and melphalan. (74 references)

6424 THE INCIDENCE OF MALIGNANT PRIMARY BONE TUMOURS IN RELATION TO AGE, SEX AND SITE.

(E.) Larsson, S. (Dep. Orthop. Surg., Univ. Umea, Sweden) and R. Lorentzon. *J Bone Joint Surg (Br)* 56B(3):534-540, 1974.

The incidence of osteogenic sarcoma, chondrosarcoma, and Ewing's sarcoma was studied among 832 malignant primary bone tumors diagnosed in 1958-68 in Sweden. Out of these, 16.2% were unspecified, 10.6% having a diagnosis based upon radiological or clinical examination only and 5.6% having a diagnosis of "sarcoma" without any further classification of the tumor. In the remaining 83.8% of the cases, the diagnosis was based upon histopathological examination of biopsy material. The three tumors showed a predilection for males. Osteogenic sarcoma showed a ratio of 1.40:1, chondrosarcoma, 1.38:1; and Ewing's sarcoma, 1.96:1. The incidence for osteogenic sarcoma and Ewing's sarcoma demonstrated a peak during adolescence. For osteogenic sarcoma, the peak occurred at a mean age of 16 yr for boys and 12 yr for girls and was associated with maximum growth velocity. Ewing's sarcoma demonstrated no sex difference regarding incidence peak. In chondrosarcoma and osteogenic sarcoma, the predominance of males over females was valid for the spinal column, pelvis, and long bones of the lower limb.

6425 POPULATION SCREENING FOR CERVICAL CANCER IN JAMAICA: RESULTS OF TWO SEPARATE SURVEYS. *(E.)* Persaud, V. (No affiliation). *West Indian Med J* 23(2):85-91, 1974.

To help define groups of women at high risk of developing cervical carcinoma, 652 apparently healthy women residing in the low-income Jamaican communities of Hermitage and August Town were cytologically examined as part of a pilot screening program. The results were compared with those obtained during a much larger screening program carried out among Jamaican family planning patients. The rate of preclinical cervical carcinoma among the women participating in the community screening program was 18 per 1,000 women tested. The younger patients were treated by cervical conization and periodic cytological follow-up and the older women were subjected to total hysterectomy with removal of a vaginal cuff. Trichomonas vaginalis organisms were seen in 126 cases, 97 of whom were under 40 yr of age. The cervix appeared healthy in 56% of the participants and showed evidence of cervicitis or erosions in 40%. Among the 36,229 women screened at the family planning clinics between 1966 and 1969, the cervix was normal in 49.3%, with evidence of cervicitis appearing in 50.1%. The prevalence of preclinical cervical carcinoma was 5.7 per 1000 women screened, the age distribution showing a peak incidence among those aged 25-29 yr. In 1969, only 20% of the patients with suspicious and positive smears returned for diagnostic biopsies and treatment. This figure increased to 40% in 1970. The results suggest that effective control of clinical carcinoma of the cervix in Jamaica may be best achieved by defining and screening other high risk groups within the general population.

6426 EPIDEMIOLOGY AND ETIOLOGY OF ESOPHAGEAL CANCER IN NORTH CHINA: A PRELIMINARY REPORT. *(E.)* Anonymous. (Coord. Group Res. Etiol. Esophageal Cancer, North China). *Chin Med J* (11): 189, 1974.

Epidemiological and etiological studies of esophageal cancer in North China were conducted from 1969-1971 and covered 181 counties and cities with a total population of nearly 50 million. The epidemiological surveys gave the following results: the average crude mortality rate was 53.96/100,000; the average mortality rats, after age and sex adjustment, was 37.39/100,000; the highest adjusted rate was 139.80/100,000 and the lowest adjusted rate was 1.43/100,000. The average incidence ration between men and women was 1.6:1. In Linhsien county, the mortality rate from esophageal cancer showed little fluctuation, the average age-sex adjusted incidence rate was 108.56/100,000 and the mortality rate was 99.76/100,000. Geographic distribution analysis showed highest rates for counties and cities in the southern part of the Taihang Mountain Range. Trace elements in the soil and drinking water, nitrosamines, secondary amines, nitrites and nitrates in food and fungus contamination were analyzed for comparative values in high and low incidence areas. Analysis of pharyngeal and esophageal cancer in domestic fowl in one high and one low incidence area showed etiologic connection between human and animal cancer. Large scale cytologic examinations of esophageal mucosa in high incidence areas revealed high frequency of epithelial hyperplasia in the adult population. Precancerous lesions and many very early subclinical esophageal cancers were recognized. The study is of value in understanding the evolution of esophageal carcinoma from the precancerous stage to carcinoma *in situ* and the invasive stages.

6427 RELIABILITY OF STATISTICAL DATA IN GASTRIC CANCER INCIDENCE. *(Hun.)* Schäfer, P. K. (Int. Dis. Clin., Justus Liebig Univ. Giessen, West Germany). *Mag Onkol* 18(1):2-8, 1974.

Considerations on the reliability of gastric cancer mortality and morbidity statistics and some results of related epidemiological surveys worldwide are presented. Mortality data are suitable for detailed analysis and for comparisons between different geographic areas after standardization for 100,000 inhabitants of the same age and sex. The reliability of mortality statistics is improving recently as a result of a considerable reduction of the incidence of unknown or erroneous etiology. Morbidity statistics considering even histologically non-verified cases are regarded to be more reliable than mortality statistics. A comparison of gastric cancer morbidity data show a time lag of 10-15 yr in the cumulation in Europe as related to Japan. Significant regional differences in the gastric cancer morbidity and mortality were found within Germany and the U.S.

6428 FAMILIAL HODGKIN'S DISEASE: AN ENVIRONMENTAL AND GENETIC DISORDER. *(E.)* Vianna, N. J. (New York State Dep. Health, Albany), J. N. P. Davies, A. K. Plan and P. Wolfgang. *Lancet* 2(7885): 854-857, 1974.

Evaluation of 46 familial cases of Hodgkin's disease among first-degree blood relations suggested that the time intervals between diagnoses are shorter than the age difference between family members. The age of the patients ranged from 14-82 yr, with 31 (67.4%)

less than 40 yr. The two prominent familial patterns were sib-sib (nine pairs) and parent-child (seven pairs). For seven of the sib pairs, the interval between diagnoses was shorter than the difference between age at diagnosis. The mean interval between diagnoses was 2.3 yr (median three yr, range 0.2-4 yr) and the mean age difference was 4.6 yr (median six yr, range 1-8 yr). The mean diagnostic interval for the seven parent-child pairs was 3.6 yr (median two yr, range 0-8.6 yr). The mean time interval between diagnoses for the four parent-child and three sib pairs living in the same household was 12 yr (median one yr, range 0-3 yr), whereas for the six sib and three parent-child pairs living apart it was 4.1 yr (median 3.3 yr, range 0.8-8.6 yr). These findings are consistent with an environmental etiology. However, many familial pairs had the same Rye subtypes at diagnosis, regardless of their relationship or proximity. Host reactivity may therefore be influenced by genetic factors.

6429 PRIMARY LUNG CANCER IN TAIWAN. *(Jap.)*

Luh, K. (Coll. Med., Natl. Taiwan Univ., Formosa), S. Kuo, C. Lin, S. Yang and K. Chen. *J Formosan Med Assoc* 73(3):129-146, 1974.

Epidemiological characteristics of primary lung cancer in Taiwan and their chonological trends were studied with analysis of available vital statistics from 1932, clinical data of 1753 histologically proved cases since 1952, and prevalence survey carried out in 1972-73. The crude mortality rate of lung cancer in Taiwan has increased 14-fold for males and 17-fold for females during the past 35 years. The increase is found to be the highest among various malignant neoplasms. The trend of high occurrence of adenocarcinoma (34.3% for males; 58.7% for females) has remained unchanged since 1952. Moreover, the incidence in cities was four times as high as that in rural areas. The smoking situation in Taiwan has not changed much during the past decades, while industrialization has been in full swing during the same period. It seems logical to assume that cigarette smoke may act as a tumor initiator and air pollution may serve as a tumor promotor.

6430 MALIGNANT NEOPLASMS OF THE THYMUS AMONG PATIENTS TREATED BETWEEN 1951-1973 AT THE INSTITUTE OF ONCOLOGY IN CRACOW. *(Pol.)*

Urbanski, K. (Inst. Oncol., Krakow, Poland). *Nowotwory* 24(3):191-200, 1974.

Between 1951-1973, 20 cases of thymus neoplasms (constituting 0.04% of all patients) were diagnosed at the Institute of Oncology in Krakow. The patients consisted of seven men and 13 women, aged 5-73 yr (average age 36.6 yr), seven were 30 yr old and there were two children aged 5 and 11 yr. In 7 cases the histological structure of the tumor could not be established. The tumors in 7 patients (1 man and 6 women) were identified as epithelial-type thymomas, in five patients (3 men and two women) as lymphoepithelial-type thymomas and in one man as a fusiform type. The neoplasms developed asymptomatically in five cases (25%) and were detected accidentally during routine radiologic studies. In other cases the most common symptom were dyspnea (ten), cough (nine), chest paint (seven) and weakness and loss of weight (six). Symptoms were present for 1-7 months, (average 3.7 months). In seven patients metastases were present at time of diagnosis. Exploratory thoracotomy and radiotherapy (2,800-4,000 r over 21-35 days) were used in eight cases, radical resection and radiotherapy in four, partial resection and radiotherapy and chemotherapy in one, and chemotherapy and radiotherapy in six. The majority (75%) died within six months of treatment, one died after two yr and two lived more than five yr. One patient is alive 7.5 yr after radical resection and radiotherapy, the other six yr after radiotherapy and chemotherapy. Both of these patients had the lymphoepithelial type of thymoma.

6431 DIFFERENTIAL DIAGNOSTICS AND SURGICAL INDICATIONS OF COLD THYROID NODULES. *(E.)*

Balázs, G. (Univ. Med. Sch., Debrecen, Hungary), S. Fazakas, L. Szikorszky, G. Hájer, G. Csáky and M. Szeleczky. *Acta Med Acad Sci Hung* 31(1-2):99-106, 1974.

The respective incidences of thyroid carcinoma, of thyroid tumors of potential malignancy and of thyroiditis were investigated on the basis of the histological features of cold nodules observed in 300 thyroids from a population of a flat area with endemic goiter. A total of 44 cold nodules proved malignant; 28 of these were differentiated and 16 were undifferentiated or sarcomatous. There was, in addition, a group of 34 patients with tumors of potential malignancy which lacked a consistent interpretation both pathologically and clinically. Thyroiditis, including its acute and chronic forms, was the source of the cold nodules in a total of 37 cases. Most tumors carried a comparatively favorable prognosis. In the group of potential malignancy, the follicular, trabecular and Hurthle-cell adenomas has a practically equal share. Of the cases of thyroditis, the majority presented an advanced destructive Hashimoto's thyroiditis, which implies a potential preblastomatous state. While in 20/40 thyroid tumors the clinical features alone had been suggestive of malignancy, in 24 the final decision for surgery was made on the grounds of the scan. The clinical parameters were designed for use in the differentiation of cold nodules and in the timing of surgery and include the relationship between cold nodule and potential malignancy; cold nodule and earlier x-ray irradiation of the cervical region; cold nodule and earlier radioiodine treatment; age and sex of the cold nodule; cold nodule and its growth; cold nodule and recurrent goiter; cold nodule and ectopic thyroid tissue in the regional lymph nodes; cold nodule and pheochromocytoma and cold nodule and calcification. It is concluded that malignant tumors of the thyroid gland are not uncommon and that the clinical parameters add to the diagnostic value of scintigraphy and allow a closer differentiation of the cold nodule.

6432 AUTORADIOGRAPHIC MEASUREMENT OF CELL PROLIFERATION IN THE DIGESTIVE TRACT. HYPOTHESES CONCERNING THE MALIGNANT DEGENERATION OF ATROPHIC MUCOSA.

(Fr.) Bleiberg, H. (Tumor Cent., Free Univ. Brussels, Belgium) and P. Galand. *Acta Gastroenterol Belg* 37(10-11):475-487, 1974.

Biopsies were performed on 30 patients with normal and pathological rectal mucosa and fragments were taken from the small intestine of ten other patients in order to study cell proliferation in comparison to that of the digestive mucosa. *In vitro* double thymidine labeling was used to determine S-phase duration, labeling index and renewal time of the cell population. Renewal time was: 90 hr for normal rectal mucosa, 34.2 hr for pathological rectal mucosa (colorectitis), 31.8 hr for normal mucosa of the small intestine, 27.9 hr for atrophied mucosa of the small intestine, 57.8 hr for normal gastric mucosa and 21.8 hr in Biermer's anemia. The renewal rate was greater for atrophic mucosa than for normal mucosa. Considering the frequency with which malignant degeneration of atrophic mucosa occurs, accelerated cell proliferation may be considered a factor favorable to malignant degeneration. The labeling index in rectal polyps was generally more elevated than that of nearby normal mucosa. This might correspond to an accelerated renewal rate in tissue with a higher cancer frequency.

6433 CELL KINETIC PATTERNS IN HUMAN ACUTE LEUKEMIA. EVIDENCE FOR CONTROL MECHANISMS.

(E.) Mauer, A. M. (Child. Hosp. Res. Found., Cincinnati, Ohio), B. C. Lampkin, C. F. Evert and N. B. McWilliams. *Bibl Haematol* (39):1014-1021, 1973.

Leukemia cell kinetics were studied using a computer simulation model in order to determine the effects cell death and the proportion of cells entering a resting phase after division might have on generation time. Using this model, both factors were found to influence the doubling time. A review of previously reported data concerning the effects of partial exchange transfusion on marrow proliferative activity and the effects of vincristine administration on cell kinetics *in vivo* and *in vitro* is presented. Exchange transfusion reduced the number of leukemic cells, as did extracorporeal irradiation of blood, but it did not result in increased marrow proliferative activity. Vincristine caused an accumulation of cells in metaphase in the first 24 hours, and by 48 hours resulted in a decrease in S phase cells. The studies suggest a true G_0 state for leukemic cells. The authors conclude that the external manipulation of cancer cell population kinetics might result in the prolonged maintenance of malignant cells in a quiescent state.

6434 THE PROLIFERATION RATE DEPENDENCE ON THE POPULATION DENSITY IN CULTURED TRANSFORMED CELLS.

(Rus.) Pletyushkin, O. (Biol. Fac., Moscow Univ., U.S.S.R.) and I. S. Tint. *Tsitologiia* 16(4):486-490, 1974.

A comparison study of the proliferation of embryonic fibroblast-like cells of a Syrian hamster and two transformed cell lines of that type was conducted by the application of autoradiography (treatment with tritium-labeled thymidine) under conditions of different cell-population densities. It was shown that the saturation density of cultures of transformed cells, where proliferation of cells would stop altogether, exceeded the corresponding saturation density of the culture of embryonic fibroblast-like cells. Inhibition of cell proliferation at high cell densities was observed for all types of cells investigated.

6435 MACROMOLECULAR SYNTHESIS AND CELL PROLIFERATION KINETICS IN THE COURSE OF INDUCTION OF PROLIFERATION IN STATIONARY CULTURES OF CHINESE HAMSTER CELLS.

(Rus.) Terskikh, V. V. (Inst. Mol. Biol. Acad. Sci. Moscow, U.S.S.R.), A. I. Zosimovskaya and M. K. Abuladze. *Tsitologiia* 16(3):317-321, 1974.

The kinetics of cell proliferation and macromolecular synthesis were studied in relation to the process of induction of proliferation by medium change in stationary cultures of Chinese hamster cells. The entry of cells of the inductive phase into the phase of DNA synthesis was observed autoradiographically. The results showed that the pre-replicative period is subdivided into two parts: (1) a transformation period in which quiescent cells transform into proliferative ones and (2) late G_1 period of the first cell cycle after the induction of proliferation. The stimulated cells resume proliferation at the maximal rate possible under the conditions of cultivation.

6436 THE RELATIONSHIP BETWEEN THE CELL POPULATION KINETICS AND CHROMOSOME PATTERN IN HUMAN TUMOURS.

(E.) Refsum, S. B. (Inst. Pathol., Univ. Oslo, Norway) and I. L. Hansteen. *Acta Pathol Microbiol Scand* [*A*] (Suppl 248):153-155, 1974.

Biopsies and chromosome analyses were conducted on tumors from patients with malignancy in the ENT region to determine the relationship between the cell population kinetics and chromosome pattern in human tumors. In some tumors the same rate of cell proliferation was found in all areas, wheras in other tumors two or three different rates of proliferation were found in different areas. The average rate of proliferation in the malignant tumors was about twice the value found in thin epithelium. The chromosome analyses of the epidermoid carcinomas showed a great spread in the chromosome numbers, both between and within the tumors. Six of the ten epidermoid carcinomas had cells with chromosome numbers around 60-70, whereas only two tumors had diploid values. In the lymphomas 3/5 cases studied had chromosome numbers around aneuploid, diploid values, whereas two other cases had a greater spread, indicating two or three different chromosome modes. In five patients with adenocarcinomas, very few cells were available for cell analyses, all having diploid, usually aneuploid values. The duration of symptoms was about the same for the epidermoid carcinomas as for the lymphomas. Between the chromosome pattern and the rate of cell proliferation in the tumors there was definite relationship. The different rate of cell proliferation and the different chromosome patterns found in one and the same tumor cannot be taken as an argument in favor of a multicellular change after carcinogenic damage. They may, however, be an indication in favor of the hypothesis that malignant change occurred in a few or probably in a single cell.

6437 LEUKAEMIC STEM CELL KINETICS IN EXPERIMENTAL ANIMALS. *(E.)* Tanaka, T. (Christie Hosp. Holt Radium Inst., Manchester, England), N. E. G. Testa and L. G. Lajtha. *Bibl Haematol* (39):984-991, 1973.

The kinetics of normal and leukemic stem cell proliferation were measured *in vitro* in unirradiated and irradiated Manchester mice. The leukemic stem line chosen was the myelomonocytic leukemia of Rf. Erythroid repopulation and ^{59}Fe incorporation were also studied. A linear relationship was found between the number of injected leukemic stem cells and the number of spleen colonies formed. Leukemia spleen clone cell (LCFU) growth was exponential between days six and eleven in unirradiated mice. LCFU growth in irradiated animals demonstrated a 12 to 24 hr lag in the spleen and femur, but no such lag was seen in the liver. Normal colony forming stem cells did not change in number for five days following leukemia injection. Erythroid repopulating capacity of bone marrow and spleen, and ^{59}Fe incorporation into red cells dropped after day five. Both normal and leukemic colonies were easily distinguishable since the leukemic colonies were large, compact dense globular masses, and the normal colonies were loose with some dense centers. Normal agar colonies forming units (nACU) in the femur declined seven days following leukemia injection, but increased after the third day in the spleen. Three phases of normal and leukemic growth are suggested based on the observed correlation between stem cell numbers and leukemia growth rates. The phases for the reaction of the normal tissue to leukemic growth are: (a) no detectable alteration (day 1-3); (b) increase in splenic nACU(days 3-6), and (c) decline in all aspects of normal hemopoiesis (day 6 onwards). The phases for leukemic growth are: (a) initial lag (0-24 hr); (b) exponential fast growth (day 1-9), and (c) declining growth (day nine to death).

6438 MORTALITY AMONG VINYL-CHLORIDE WORKERS. *(E.)* Falk, H. (Cent. Dis. Control, Atlanta, Ga.), C. W. Heath, Jr., C. D. Carter, J. K. Wagoner, R. J. Waxweiler and W. T. Stringer. *Lancet* (7883):784, 1974.

6439 EPIDEMIOLOGY OF GASTROINTESTINAL CANCER. *(E.)* Selikoff, I. J. (Environ. Sci. Lab., Mt. Sinai Sch. Med. City Univ. New York, N.Y.). *Environ Health Perspect* 9:299-305, 1974.

6440 KINETIC CHANGES IN CELL CLONES OF A HUMAN MELANOMA *IN VITRO*. *(It.)* Raffetto, G. (Dep. Oncol., Univ. Genoa, Italy), C. M. Parodi, G. Marino, R. Troiano and L. Santi. *Pathologica* 66(961/962):505-512, 1974.

6441 THE INCIDENCE OF MALIGNANT TUMORS IN PATIENTS WITH SARCOIDOSIS. *(Dan.)* Brincker, H. (Danish Inst. Clin. Epidemiol., Copenhagen, Denmark) and E. Wilbek. *Ugeskr Laeger* 136(39):2192-2195, 1974.

6442 TEMPORAL-SPATIAL DISTRIBUTIONS OF LEUKAEMIA AND HAEMATOSARCOMA IN SEVEN FRENCH REGIONS. *(E.)* Alperovitch, A. (Stat. Res. Unit, Villejuif, France), C. Hesse, P. Lazar, M. Hayat and R. Flamant. *Int J Epidemiol* 3(3):209-218, 1974.

6443 EPSTEIN-BARR (EB) VIRUS INFECTION IN BRAZIL. *(E.)* Jamra, M. (Inst. Hematol., São Paulo, Brazil). *Medicina (B Aires)* 34(Suppl. 1):33-34, 1974.

6444 BRONCHOGENIC CARCINOMA IN KUWAIT. *(E.)* Ghalayini, G. (Chest Diseases Hosp., Kuwait). *J Kuwait Med Assoc* 8(1):3-8, 1974.

6445 CANCER EPIDEMIOLOGY IN INDIA: A CRITIQUE. *(E.)* Sanghvi, L. D. (Cancer Res. Inst., Tata Mem. Cent., Bombay, India). *Indian J. Med Res* 62(12):1850-1870, 1974.

6446 EPIDEMIOLOGICAL ASPECTS OF CYTOLOGICAL DETECTION OF EARLY CERVICAL CARCINOMA. *(E.)* Kovacic, J. (Clinical Hosp., Ljubljana, Yugoslavia). *Bull Sci Cons Acad Sci Arts R. S. F. Yougosl Sect A* 19(7/8):192, 1974.

6447 THE ROLE OF CHEMICALS IN HUMAN CANCER: OCCUPATIONAL FACTORS. *(E.)* Murray, R. (Trades Union Congr., London, England). *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:111-114, 1974.

6448 SOME ORGANOCHLORINE PESTICIDES. *(E.)* Anonymous. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man* 5:1-241, 1974.

6449 THE DYNAMICS OF THE POPULATION STRUCTURE OF ZAJDELA ASCITES HEPATOMA DURING GROWTH AND METASTASIZING (CYTOSPECTROPHOTOMETRIC STUDY). *(Rus.)* Kiseleva, N. S. (Inst. Exp. Clin. Oncol., Moscow, USSR), A. I. Pavlotskii, E. L. Vendrov, Iu. E. Morozov and B. B. Fuks. *Tsitologiia* 16(7):879-885, 1974.

6450 EXPOSURE TO HERBICIDES: MORTALITY AND TUMOR INCIDENCE. *(Sw.)* Axelson, O. (Dept. Ind. Med., Reg. Hosp., Orchro, Sweden), M. Rehn and L. Sundell. *Lakartidningen* 71(24):2466-2470, 1974.

6451 EPIDEMIOLOGY OF TUMORS AND TUMOR-LIKE LESIONS OF BONE IN CHILDHOOD AND ADOLESCENCE. *(Ger.)* Fodisch, H. J. (Inst. Child Pathol., Univ. Bonn, West Germany), I. Krainer, W. W. Hopker and G. Mikuz. *Verh Dtsch Ges Pathol* 58:441-444, 1974.

6452 TUMOR INCIDENCE IN THE CZCZECIN REGION. *(Pol.)* Staszewski, J. (Inst. Oncol., Gliwice, Poland) and J. Swiecki. *Nowotwory* 24(4):262-275, 1974.

6453 EPIDEMIOLOGICAL STUDIES OF MALIGNANT NEOPLASMS IN THE UPPER RESPIRATORY TRACT IN THE UKRAINE. *(Rus.)* Samokhodskii, V. N. (Kiev Res. Inst. Otolaryngol., USSR). *Zh Ushn NOs Gorl Bolezn* (2):76-80, 1974.

6454 THE USE OF LIQUID DISINTEGRATION TECHNIQUE FOR THE QUANTITATIVE EVALUATION OF CELL ADHESION IN TISSUES. *(Rus.)* Arkhipenko, V. I. (Dnepropetrovsk Med. Inst., USSR), G. A. Chuich and V. F. Ushakov. *Tsitologiia* 16(4):491-496, 1974.

6455 MORTALITY IN THE OTOLARYNGOLOGICAL HOSPITALS IN THE UKRAINE (1969-1970). *(Rus.)* Zaritskii, L. A. (Kiev Res. Inst. Otolaryngol., USSR) and V. G. Kryzhanova. *Zh Ushn Nos Gorl Bolezn* (5):86-90, 1974.

6456 BREAST CANCER MORTALITY IN THE DNEPROPETROVSK REGION. *(Rus.)* Logvinenko, I. A. (Dnepropetrovsk Med. Inst., USSR). *Vopr Onkol* 20(11):63-66, 1974.

6457 EPIDEMIOLOGY OF MALIGNANT MELANOMA OF THE SKIN. *(Ger.)* Berndt, H. (Cent. Inst. Oncol. Res., Berlin-Buch, East Germany). *Arch Geschwulstforsch* 44(3):267-277, 1974.

See also:

* (Rev): 6005, 6026, 6038, 6045, 6060, 6069, 6076
* (Immun): 6361
* (Path): 6380, 6398

6458 ERROR RATES IN CERVICAL CYTOLOGICAL SCREENING TESTS. *(E.)*

May, D. (Dep. Community Med., Univ. Manchester, England). *Br J Cancer* 29(2): 106-113, 1974.

In an attempt to clear up the confusion in the literature concerning the interpretation of cervical cytological screening tests, the principle on which the data acquired in a series of tests should be tabulated is stated. Seven rates relevant to cervical cytological testing are defined, five of which concern the occurrence of errors. Three of these rates, the true positive rate, the true rate of false positives, and the true rate of false negatives, are of fundamental importance, whereas the remaining four are of secondary importance. The interrelations of six of the seven rates are expressed as equations; and selected entries from a comprehensive set of conversion tables, constructed by means of the equations, are used to exemplify trends among the numerical values of the rates. The direct derivation of the rates is illustrated by an example taken from the literature, which also provides instances of error rates which are large, and of considerable contrasts between error rates differently defined.

6459 PART I. EPIDERMAL GROWTH FACTOR: CHEMICAL AND BIOLOGICAL CHARACTERIZATION. *(E.)*

Cohen, S. (Vanderbilt Univ. Sch. Med., Nashville, Tenn.). *Recent Prog Horm Res* 30:533-550, 1974.

Partially purified extracts of submaxillary glands of adult male mice caused precocious opening of the eyelids and incisor eruption when injected s.c. into newborn mice. This biological activity was due to enhancement of epidermal growth and keratinization by a polypeptide designated epidermal growth factor (EGF). The chemical and physical properties of EGF are described and its biological activity discussed. Gel filtration of aqueous extracts of the submaxillary glands yielded an EGF with a molecular weight of approximately 74,000. This high-molecular weight EGF can be reversibly dissociated into two molecules of low-molecular weight EGF (6400) and two molecules of an EGF-binding esterase. The low-molecular weight EGF and the EGF-binding esterase can be recombined to form a high-molecular weight complex. EGF possesses a carboxyl-terminal-arginine residue, suggesting that it may be generated from a precursor protein by possible proteolytic action of the EGF-binding esterase. The eyelid-opening effect in newborn rats is demonstrable at a dose level of 0.1 μg/g/day. At higher doses (1-2 μg/g/day), EGF results in distinct growth inhibition and fatty liver. In cultures of chick embryo epidermis, EGF stimulates total net protein and RNA synthesis and the conversion of preexisting ribosomal monomers to functional polysomal structures; it also induces a marked but transient increase of ornithine decarboxylase activity. The concentration of the factor in the submaxillary gland is dependent on the hormonal status of the animal and its synthesis in the gland may be a specific function of tubular cells. Secretion of EGF in mouse serum is stimulated by α-adrenergic compounds. Although there is no direct evidence for the role of the growth factor in normal development and cell control, these observations indicate that an important function does exist.

6460 REGULATION OF GROWTH *IN VITRO*. I. CONTROL OF ORNITHINE DECARBOXYLASE LEVELS IN UNTRANSFORMED AND TRANSFORMED MOUSE FIBROBLASTS BY SERUM. *(E.)*

Lembach, K. J. (Vanderbilt Univ. Sch. Med., Nashville, Tenn.). *Biochim Biophys Acta* 354(1): 88-100, 1974.

The level of ornithine decarboxylase activity was compared in normal and simian virus 40 (SV40)-transformed mouse 3T3 cells grown under various conditions. A rapid and marked increase in the activity of this enzyme was induced by the addition of serum to cultures limited in their proliferation by prior incubation in media containing low serum concentrations; both the normal and transformed cells were serum-dependent, but the normal cells required higher serum concentrations for growth. The increase in enzyme activity following the addition of serum to the medium was prevented by inhibitors of [illegible] synthesis (actinomycin D and cycloheximide [illegible] enzyme in both cell types exhibited a high [illegible] rate that was not substantially altered under varying growth conditions. In addition, the steady-state and induced levels of ornithine decarboxylase [illegible] varied with the extracellular concentration [illegible] serum in a characteristic manner for normal as compared with transformed cells. A close correlation between the level of this enzyme and cell proliferation [illegible] observed in each cell type. Gamma [illegible] serum elicited a very poor induction of the enzyme [illegible] the normal 3T3 cultures, but it significantly increased the enzyme level in the transformed cells; [illegible] both cases, however, the effect was less [illegible] than that achieved with whole serum. The results indicate the importance of ornithine decarboxylase for growth in culture and suggest that the increase in decarboxylase activity caused by serum does not result from changes in the rate of enzyme degradation.

6461 GROWTH CONTROL OF DIFFERENTIATED FETAL RAT HEPATOCYTES IN PRIMARY MONOLAYER CULTURE. VI. STUDIES WITH CONDITIONED MEDIUM AND ITS FUNCTIONAL INTERACTIONS WITH SERUM FACTORS. *(E.)*

Koch, K. (Cell Biol. Lab., Salk Inst., San Diego, Calif.) and H. L. Leffert. *J Cell Biol* 62(3):780-791, 1974.

Conditioned medium (CM) harvested from a primary monolayer cultures of differentiated fetal rat hepatocytes was studied to determine the manner by which it regulated growth of these cells. Serum-deficient (≤ 0.00003% vol/vol) CM initiated DNA synthesis and mitosis in homologous quiescent cultures, while CM similarly prepared from 3T3 fibroblast cultures was inactive. At least two conditioning factors were involved in initiating DNA synthesis. The first was arginine; it was obligatory, synthesized by the cells and released into the culture medium. The second, a lipid or lipid-containing material was stable to pH extremes (pH 2, pH 10) and chromatographed with an apparent $R_f \sim 0.5$ on silica gel thin-layer plates using hexane-ether (4:1) as the solvent system. The time that these cultured hepatocytes entered or left the G_0 or early G_1 phase of the cell cycle was determined in part by their capacity to use available conditioning factor and nutrient components of the medium, in particular arginine. Serum factors including serum fraction I, insulin, and possibly lipid-like conditioning material appeared to initiate DNA synthesis by controlling cellular processes involved with the enhanced utilization and synthesis of growth-limiting nutrients.

6462 GROWTH CONTROL OF DIFFERENTIATED FETAL RAT HEPATOCYTES IN PRIMARY MONOLAYER CULTURE. VII. HORMONAL CONTROL OF DNA SYNTHESIS AND ITS POSSIBLE SIGNIFICANCE TO THE PROBLEM OF LIVER REGENERATION.

(E.) Leffert, H. L. (Cell Biol. Lab., Salk Inst., San Diego, Calif.). *J Cell Biol* 62(3): 792-801, 1974.

The initiation of DNA synthesis in quiescent primary cultures of fetal rat hepatocytes was studied using various culture media containing known hormones. Media containing crystalline insulin (0.01-10 μg) or 20,000-fold purified somatomedin (0.01-1.0 μg/ml) stimulated DNA synthesis. Growth hormone (0.025 μg/ml), hydroxycortisone (0.025 μg/ml), and guanosine 3':5'-cyclic monophosporic acid (10^{-5} M) failed by themselves to initiate DNA synthesis, but added to insulin enhanced the stimulatory effect of the latter by 50-100%. Thyroid hormones (L-T_3, L-T_4, 7.5-30 ng/ml) also had no effect by themselves, but when they were added to thyroid hormone-depleted serum, the reconstituted mixtures showed an enhanced capacity to initiate DNA synthesis. In contrast, glucagon (0.01 μg/ml) inhibited the insulin-stimulated DNA response by about 50% without reducing basal DNA synthesis rates. The results combined with previously reported data indicate that the environmental control over the initiation of DNA synthesis is complex and can involve the participation of numerous factors. The *in vitro* findings are consistent with the concept that liver regeneration is hormonally controlled and suggest that alterations in the intrahepatic ratio of insulin to glucagon may be growth regulatory.

6463 DIFFERENTIATION OF TUMOR CELLS: INDUCTION OF ERYTHROCYTE MEMBRANE-SPECIFIC ANTIGENS IN FRIEND LEUKEMIA CELLS.

(E.) Sugano, H. (No affiliation), M. Furusawa, T. Kawaguchi and Y. Ikawa. *Recent Results Cancer Res* 44:30-44, 1974.

The differentiation of Friend leukemia cells along the erythrocytic series was studied *in vivo* and *in vitro*. The conditions and substances which enhance the erythroid differentiation of Friend cells were examined using the fluorescein antibody technique for erythrocyte membrane-specific antigens (MEM-antigens) as the characteristic marker of an erythroid cell. The induction of MEM-antigens in Friend cells was studied in cells cultured in the peritoneal cavities of mice syngeneic to the Friend cells and of rats allogeneic to the Friend cells. *In vitro*, various substances and conditions which enhance erythrocytic expression were studied. MEM-antigens were produced in large quantities in primary Friend cells when the Friend disease occurred *in vivo*. After long-term culture, most of the Friend cells lost the ability to synthesize MEM-antigens but still maintained some slight erythrocytic gene expression. The MEM-antigens in the producing cells were distributed on the cell surface and diffusively in the cytoplasm and sometimes in the perinuclear area. Erythrocytic maturation of the Friend cells was enhanced *in vivo*, this enhancement of erythrocytic expression being greatly exaggerated in an allogeneic animal. The *in vivo* experiments suggest a close relationship between virus particles and erythrocytic expression, but this relationship was not clear in the *in vitro* experiments. The *in vitro* experiments showed that the induction or enhancement of MEM-antigens could occur in the presence of inhibitors of DNA synthesis (cytosine arabinoside, 5-fluorodeoxyuridine, N-hydroxyurea, bleomycin, and Mitomycin-C) and various other substances and conditions specific to Friend cells (DMSO, N-dimethylrifampicin, culture in diffusion chambers, and a combination of erythropoietin, VB_{12}, and $(But)_2$-cAMP). The results suggest that the expression of the viral genome incorporated into a cell is not directly related to the differentiation of the Friend cells.

6464 MOBILITY OF LECTIN SITES ON THE SURFACE MEMBRANE AND THE CONTROL OF CELL GROWTH AND DIFFERENTIATION.

(E.) Sachs, L. (Dep. Genet., Weizmann Inst. Sci., Rehovot, Israel), M. Inbar and M. Shinitzky. *Cold Spring Harbor Conf Cell Proliferation* 1:283-296, 1974.

Experimental data are presented on the mobility of concanavalin A (ConA) binding sites on surface membranes and on membrane fluidity of specific sites as related to regulation of cell growth in normal and transformed fibroblasts, normal lymphocytes and lymphoma cells, and to the normal differentiation of myeloid leukemic cells to macrophages and granulocytes. Also included are data on the mobility of sites for wheat germ and soybean agglutinins on normal lymphocytes. The mobility of carbohydrate-containing structures associated with binding sites for ConA differs in normal and malignant tissue. In cells (fibroblasts) that form a solid tissue *in vivo*, the transformation of normal into malignant cells is associated with an increase in membrane fluidity of the carbohydrate-containing structures. However, in cells (lymphocytes) that are in suspension *in vivo*, the malignant transformation is associated with a decreased fluidity of these structures on the surface membrane. This increased fluidity of specific sites on the membrane in transformed fibroblasts can explain their lack of contact inhibition, their ability to grow in soft agar, and their malignancy in a solid tissue. In normal lymphocytes, ConA sites have a higher degree of mobility than the sites for wheat germ and soybean agglutinins. These two lectins, in contrast to ConA, also do not activate normal lymphocytes to undergo DNA synthesis. Results also showed a difference in the fluid state of ConA binding structures on the surface membrane in myeloid leukemic cells that can be induced to differentiate normally (D^+cells) and myeloid leukemic cells that could not be induced to differentiate (D^- cells). A higher degree of fluidity was associated with a gain in the ability of myeloid leukemic cells to undergo normal differentiation to mature macrophages and granulocytes. The increased fluidity in D^+ cells was associated with an increased migration of the differentiated cells in soft agar. Differences in membrane fluidity of specific sites may explain differences in the ability of cells to respond to other differentiation-inducing stimuli, in the cellular response to hormones, and differences in cell migration in embryonic development and carcinogenesis.

6465 Po_2 DISTRIBUTION IN TUMOR TISSUE OF DS-CARCINOSARCOMA. *(E.)* Vaupel, P. (Inst. Physiol., Univ. Mainz, West Germany) and G. Thews. *Oncology* 30(6):475-484, 1974.

Critical oxygen supply conditions in tumor tissue of DS-carcinosarcoma were investigated. Polarographic measurements of local O_2 partial pressures in DS-carcinosarcoma revealed that in superficial tissue layers, the Po_2-profiles have a characteristic course which quantitatively can be attributed to the diffusion of the atmospheric oxygen into the tissue. A monotonous pattern of very low Po_2 values predominated in the tissue DS-carcinosarcoma. Only relatively slight regional differences were found. The Po_2-gradients were almost completely uniform and very flat. During tumor growth the mean O_2 pressure decreased exponentially from 13.6 mm Hg to 3.0 mm Hg. During this gain in weight the Po_2 values ranging from 0 to 5 mm Hg increased from 50-80%. In major parts of the tumor tissue the actual Po_2 was below a critical value so that the activity of the respiratory enzymes was reduced. Investigations of the dynamic behavior during respiratory hyperoxia showed in most cases that the Po_2 elevations in the tumor tissue were very small. Sometimes there was no response at all. Oxygen pulses only showed significant Po_2 changes when the initial Po_2 values were relatively high. The time constants of O_2 responses after sudden changes of arterial Po_2 depended on the initial Po_2 values in the tumor tissue. Sufficiently supplied cells in the direct neighborhood of the capillaries had a relatively high O_2 consumption rate which exceeded the global mean and caused sharp pressure decreases so that only low O_2 pressures were available for more distant cells. The results of previous studies are confirmed by these investigations.

6466 SOME EFFECTS OF DIBUTYRYL CYCLIC ADENOSINE MONOPHOSPHATE (DBCAMP) ON RABBIT BONE MARROW CELLS IN CULTURE: CELL CLUMPING AND CHOLINESTERASE PRODUCTION. *(E.)* Garry, V. F., Jr. (U.S. Army Biomed. Lab., Edgewood Arsenal, Aberdeen Proving Ground, Md.), L. W. Harris and J. P. Petrali. *Oncology* 30(6):517-528, 1974.

The effects of dibutyryl cyclic adenosine monophosphate (DBcAMP) on rabbit bone marrow cells cultured *in vitro* for 24 hr were investigated. Marrow cultivated in the presence of 0.001 M DBcAMP formed large dense cell clusters, while those from control cultures formed smaller more discrete cell clusters. Actinomycin D (1×10^{-7} M) prevented cluster formation. Treatment with DBcAMP had no effect on cell viability, but produced a reduction in the number of mature cells as compared to control untreated preparations during the same time interval. Erythroid cell maturation was inhibited in the presence of DBcAMP; cholinesterase synthesis was enhanced. Most of the cells containing newly synthesized membrane-bound cholinesterase were erythroid. It is suggested that the cyclic nucleotide may stimulate membrane protein synthesis. It also promotes cell differentiation (organic formation) and probably therefore inhibits erythroid cell maturation which is a kind of dedifferentiation.

6467 LEUKEMIC CELL PROLIFERATION AND MATURATION *IN VITRO*. *(E.)* Robinson, W. A. (Univ. Colorado Med. Cent., Denver), M. A. Entringer and A. Otsuka. *Bibl Haematol* (39):1030-1036, 1973.

Peripheral blood and bone marrow cells obtained from normal and leukemic patients were studied for maturation and proliferation in tissue culture. Cells from patients with acute granulocytic leukemia (AGL) will divide and establish colonies in the presence of normal white blood cell (WBC) feeder layers, although these colonies are smaller than those established by normal marrow cells. It was concluded that these were colony forming cells because of a linear correlation found between the number of colonies and the number of leukemic cells plated. Peripheral blood cells from leukemia patients did not serve as feeder layers for normal or leukemic cells. They did not inhibit the activity of normal WBC feeder layers indicating that they do not secrete an inhibitory substance. N-ethylmaleimide and freezing both inhibited the colony stimulating activity of normal WBCs. Cycloheximide did not inhibit the WBC colony stimulating activity. It is stated that WBC feeder layers stimulate colony growth by active secretion of a stimulating factor(s) and it is suggested that the major defect of acute granulocytic leukemia cells may lie in their inability to make and release the factor(s) that are needed for normal cell maturation.

6468 TESTICULAR AND PROSTATIC CYCLIC AMP METABOLISM FOLLOWING CHRONIC CADMIUM TREATMENT AND SUBSEQUENT WITHDRAWAL. *(E.)* Sutherland, D. J. B. (Fac. Med., Univ. Ottawa, Canada), B. K. Tsang, Z. Merali and R. L. Singhal. *Environ Physiol Biochem* 4(5):205-213, 1974.

The effects of daily i.p. injection of cadmium chloride (1 mg/kg) for 45 days on prostatic and testicular weights of mature male Sprague-Dawley rats were investigated. Cyclic AMP levels in the prostrate gland were reduced to 57% of normal values, which appeared to be due to the decrease in adenyl cyclase activity since cyclic AMP metabolism by phosphodiesterase was not significantly altered. Cyclic AMP binding to prostatic protein kinase was increased following treatment, as was the activity of the cyclic AMP-dependent form of protein kinase. Testicular adenyl cyclase was stimulated by the treatment. The endogenous cyclic AMP levels remained unaffected since the increase in testicular adenyl cyclase was offset by a concomitant increase in the activity of phosphodiesterase. Although the activities of the cyclic AMP-dependent and the independent forms of testicular protein kinase were significantly depressed, the binding of cyclic AMP to protein kinase from testes of Cd-treated rats was not affected. Discontinuation of treatment for 28 days resulted in a reversal of several of the Cd-induced changes in cyclic AMP metabolism of the prostate gland, but the weight of the glands remained essentially in the same range. Cessation of Cd treatment restored adenyl cyclase and protein kinase activities in the testes to normal. Endogenous cyclic AMP levels, the cyclic AMP binding capacity of protein kinase, as well as testicular phosphodiesterase and the cyclic AMP-independent form of protein kinase were still significantly reduced in the withdrawal group. The data suggest that cyclic AMP metabolism in both the primary and the seconday reproductive organs is thus altered and that the metabolic changes persist following termination of their daily exposure to this heavy metal.

6469 SYNTHESIS OF PLASMA PROTEINS BY NORMAL AND MALIGNANT HEPATOCYTES IN CULTURE. *(Fr.)*

Rioche, M. (Natl. Lab Vet. Res., Nice, France), S. Quelin and R. Masseyeff. *Pathol Biol (Paris)* 22(10): 867-876, 1974.

Cell cultures were made from 19 cases of primary liver cancer, eight normal human livers, and the noncancerous regions of three livers hosting primary liver cancer. The cultures were grown in media containing amino acids marked with ^{14}C for identification of synthesized proteins by radio-immunoelectrophoresis and in unmarked media for identification of synthesized proteins by double immunodiffusion against specific antisera. The plasma proteins that were investigated were albumin, fibrinogen, haptoglobin, transferrin, β-lipoprotein, β-2-glycoprotein I, and α-fetoprotein. The cultured cells were of three types: hepatocytes, fibroblastoids, and fibroblasts. Protein synthesis was greater for cultures with a higher proportion of hepatocytes and showing greater proliferative activity. Of the 19 cell cultures of cancerous hepatocytes and the eight cell cultures of normal hepatocytes, the numbers of cultures showing synthesis of albumin were, resp., 19 and 7; of transferrin, 18 and 6; of fibrinogen, 16 and 3; of hepatoglobin, 12 and 5; of β-lipoprotein, 6 (out of 11 assays) and 1; of β-2-glycoprotien I, 15 and 2; and of α-fetoprotein, 15 and 0. Thus the relative amounts of protein synthesis for the different proteins is about the same for cancerous and healthy cells, except for β-2-glycoprotein and α-fetoprotein. Semiquantitive study of protein synthesis showed that cancerous cells secreted the greatest amount of proteins, healthy cells from a liver that hosted a primary liver cancer secreted less protein, and hepatocytes from a healthy liver produced the least amount of protein.

6470 PHYSICAL PROTEIN REGULATION OF ADENYLATE KINASES FROM MUSCLE, LIVER, AND HEPATOMA.

(E.) Criss, W. E. (Univ. Florida Coll. Med., Gainesville), T. K. Pradhan and H. P. Morris. *Cancer Res* 34(11):3062-3065, 1974.

Homogenous preparations of the major adenylate kinases (EC 2.7.4.3) from rat liver, skeletal muscle, and Morris hepatoma 3924A were compared by equilibrium ultracentrifugation, sodium dodecyl sulfate:polyacrylamide gel disc electrophoresis, amino acid analysis, and two-dimensional polypeptide mapping. The hepatoma enzyme is composed of two polypeptide subunits of 13,000 daltons each and has a native molecular weight near 24,000 daltons. Liver adenylate kinase consists of two 13,000 dalton subunits and an 11,000 dalton subunit; the native form exists near 40,000 daltons. The muscle enzyme has four subunits at 13,000 daltons each. Its native form is near 50,000 daltons. A unique polypeptide sequence in the liver adenylate kinase is also indicated from "fingerprint" and amino acid analysis. It is probable that all three forms of adenylate kinase have subunits with similar size, but the liver form also has a unique smaller subunit that may be involved in its regulatability by citric acid-cycle metabolites. This unique polypeptide subunit is not present in the heptoma enzyme. Therefore, if this latter polypeptide is produced from the depression of a single structural gene, loss of the functioning of this gene in the tumor does not necessarily mean loss of enzymatic activity; it probably indicates a loss in ability of enzymatic activity to regulate metabolites.

6471 POLY(ADP-RIBOSE) SYNTHESIS AND CELL DIVISION.

(E.) Kidwell, W. R. (Natl. Cancer Inst., Bethesda, Md.) and K. E. Burdette. *Biochem Biophys Res Commun* 61(2):766-773, 1974.

A clone of HeLa cells was selected in the presence of 5-methylnicotinamide, and the growth of these cells was compared with that of normal cells to determine whether poly(adenosine diphosphate-ribose) synthesis is obligatory for cell growth and division. In the presence of 10 mM 5-methylnicotinamide, the resistant cells grew at 70% of the rate of the same cell culture without 5-methylnicotinamide. Growth of normal HeLa cells was completely inhibited by 10 mM 5-methylnicotinamide. In the absence of 5-methylnicotinamide, the two cell types had the same doubling time (approximately 24 hr). Poly(adenosine diphosphate-ribose) synthesis in resistant cell nuclei was less sensitive to 5-methylnicotinamide (0.1-10.0 mM) than comparable synthesis in the nuclei of sensitive cells. At 1.0 mM 5-methylnicotinamide, nicotinamide adenine dinucleotide incorporation was reduced by 63% in resistant cell nuclei and 92% in sensitive cell nuclei. These results indicated that poly(adenosine diphosphate-ribose) synthesis in intact cells is obligatory for cell growth and division. Protein synthesis in HeLa cells was inhibited by pronase (100 μg/ml) or trypsin (500 μg/ml), suggesting that inhibition of protein synthesis is membrane-mediated *in vitro* as well as *in vivo*.

6472 INCORPORATION OF RADIOACTIVE OROTIC ACID AND 8-AZAGUANINE INTO RAT LIVER RNA. *(E.)*

Pfeifer, G. D. (Nutr. Inst., U.S. Dep. Agric., ARS, Beltsville, Md.), O. E. Michaelis, IV and B. Szepesi. *Res Commun Chem Pathol Pharmacol* 9(4):779-782, 1974.

Specific pathogen-free male Wistar rats were injected i.p. with ^{3}H-orotate or 2-^{14}C-8-azaguanine; unlabeled 8-azaguanine (8AG) had been injected one hr previously. Three hr after the injection of the labeled compounds, the animals were killed and the incorporation of ^{14}C-8AG and 3H-orotic acid into the liver RNA compared. The fractional rates of incorporation of the two compounds into the liver were nearly equal, this being true in both continuously fed and starved-refed fats. However, the starved-refed rats accumulated more ^{3}H into the liver RNA than did the continuously fed rats. This difference was particularly apparent when the accumulation of label was expressed/standard rat (100 g body weight). Liver RNA labeled with ^{14}C-8AG, hydrolyzed, and coprecipiated with unlabeled 8AG gave a constant specific activity. This was interpreted as indicating that the ^{14}C label was incorporated at ^{14}C-8AG. The incorporation of ^{3}H-orotic acid was not decreased by the presence of 8AG. The use of 8AG in whole animal studies, therefore, appears both feasible and well justified.

6473 ISOLATION OF A MAJOR CELL SURFACE GLYCOPROTEIN FROM FIBROBLASTS. *(E.)*

Yamada, K. M. (Dep. Biol., Univ. Oregon, Eugene) and J. A. Weston. *Proc Natl Acad Sci USA* 71(9): 3492-3496, 1974.

Secondary chick embryo fibroblast cultures and primary chick heart fibroblast cultures (extracted with serum-free medium) released proteins which could be concentrated and run on polyacrylamide gels. The yield of the major band, a cell surface protein (CSP), was increased considerably by including 0.2 M urea in the extraction medium. The extracted protein and the identified surface protein component seen in gels of cell homogenates were identical. The CSP was most probably a glycoprotein of high molecular weight (220,000). It apparently is either a highly asymmetric molecule or is aggregated, and it sediments as a particle of less than 20S. The CSP was synthesized by cells, but was not identifiable as collagen. The amount of CSP recovered increased linearly with increasing cell density. Increasing cell interaction may facilitate the release of CSP. It is suggested that the isolation of this CSP may now provide a means of determining its function.

474 MODIFIED TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS SYSTEMS FOR HIGHER MOLECULAR EIGHT NONHISTONE CHROMATIN PROTEINS FROM NORMAL RAT IVER AND NOVIKOFF HEPATOMA ASCITES CELLS. *(E.)*

usch, G. I. (Baylor Coll. Med., houston, Tex.), C. Yeoman, C. W. Taylor and H. Busch. *Physiol hem Phys* 6(1):1-10, 1974.

nadequate resolution in the high molecular weight region by two-dimensional electrophoresis led to modification of the system by decreasing gel concentrations. Nuclei from normal livers of Holtzman rats and Novikoff hepatoma ascites cells were used. The chromatin was isolated, digested with DNase I and the nonhistone fraction II was clarified by centrifugation. The first dimension disc gel was composed of: 6% acrylamide, 0.21% bisacrylamide, 0.1% ammonium persulfate, 1% TEMED (N,N,N',N'-tetramethylethylenediamine), 1.8 N acetic acid and 4.5 M urea. The second dimension used both 8% acrylamide (with 0.21% bisacrylamide, 0.05% TEMED, 0.1% SDS, 0.075% persulfate, 6 M urea and 0.1 M phosphate buffer) and an 8:12% acrylamide linear gradient. The first dimensional gel was polymerized in a cap gel of the same concentrations as the 8% except that it contained 0.01 M phosphate. The 8:12% linear gradient gel was prepared with an identical cap gel to that described above. In the second dimension gradient gels decreased migration of the slower proteins while the larger ones continued to enter the gel pattern. The new system increased resolution for previously described spots in the intermediate and high weight regions and gave evidence of a larger number of spots in the high molecular weight region for the ascites cells.

6475 BIOGENIC AMINES IN CULTURED NEUROBLASTOMA AND ASTROCYTOMA CELLS. *(E.)*

Narotzky, R. (Northwestern Univ. Med. Sch., Chicago, Ill.) and W. Bondareff. *J Cell Biol* 63(1):64-70, 1974.

The catecholamine content of a cloned line of neuroblastoma cells (derived from mouse neuroblastoma NB-2a) and two clones of an astrocytoma cell line (C6 and C6P) were studied *in vitro*. Biogenic amines were localized by the fluorescence histochemical method, isolated electrophoretically after labeling with radioactive precursors, and quantitated by spectrophotofluorometric assay. Incubation in media containing L-(^{14}C)tryptophan for 24 hr, followed by high-voltage electrophoresis, radiochromatogram scanning, and scintillation counting, confirmed the presence of (^{14}C)dopamine, and (^{14}C)-norepinephrine, (^{14}C)epinephrine, (^{14}C)serotonin, (^{14}C)tyramine, and (^{14}C)octopamine. Dopamine, norepinephrine, epinephrine, and serotonin were demonstrated in concentrations of 1.19, 0.027, 0.038, and 0.148 μg/mg protein, resp., within cells in a stationary growth phase. Fluorescence-microscope histochemistry also indicated the presence of various biogenic amines in the cultured astrocytoma cells. The average concentrations of dopamine, norepinephrine, epinephrine, and serotonin were 0.25, 0.012, 0.021, and 0.019 μg/mg, resp., in the C6 cells, and 0.45, 0.024, 0.040, and 0.04 μg/mg resp., in the C6P cells. The NB-2a and C6 cells differed significantly in their concentrations of all four biogenic amines, and the NB-2a cells and C6P cells differed significantly in their concentrations of dopamine and serotonin. The two clones of astrocytoma cells also differed significantly in their concentrations of all four biogenic amines. The results suggest that cloned lines of NB-2a and astrocytoma cells synthesize monoamines and that there are at least two cell populations in the NB-2a clone, one synthesizing primarily catecholamines, and the other synthesizing primarily serotonin.

6476 EFFECT OF NERVE GROWTH FACTOR ON THE EXPRESSION OF COLCHICINE-BINDING ACTIVITY AND 14-3-2 PROTEIN IN AN ESTABLISHED LINE OF HUMAN NEUROBLASTOMA.

(E.) Kolber, A. R. (Washington Univ. Med. Sch., St. Louis, Mo.), M. N. Goldstein and B. W. Moore. *Proc Natl Acad Sci USA* 71(10): 4203-4207, 1974.

Cultured human neuroblastoma cells (NJB cells) were incubated with highly purified nerve growth factor (NGF) (1 U/ml), and the effect of the protein on cell growth, colchicine-binding activity, and the expression of 14-3-2 protein (a characteristic of differentiated neural cells) was determined. NGF induced the outgrowth of neurites from the cultured NJB cells, along with a concomitant increase in colchine-binding activity in culture extracts. The parallel stimulation of neurite outgrowth from the cells and colchicine-binding activity in the extracts was interpreted to represent the *de novo* synthesis of microtubular subunit protein in response to the challenge by NGF. The regulation of the expression of 14-3-2 protein was not affected by the addition of NGF. The concentration of 14-3-2 protein expressed in the tumor cells was about the same as that in the adult human brain (about 0.2% of the soluble protein). The results indicate that tubulin represents about 10% of the total soluble protein at the time of maximum concentration and that tubulin production and the expression of 14-3-2 protein, both characteristic expressions of differentiated neural cells, are independently regulated in NJB neuroblastoma cells.

6477 TRANSFER-RNA METHYLASE ACTIVITY IN RAT ASCITES HEPATOMAS: ALTERED METHYLATION PATTERNS IN THE PRESENCE OF GUANIDINE CARBONATE.

(E.) Iwanami, Y. (Dep. Chem., Sasaki Inst., Tokyo, Japan) and M. Inose. *Gann* 65(6):481-492, 1974.

In vitro methylation of *Escherichia coli* t-RNA by enzyme preparations from hepatoma AH-108A cells and normal liver was studied by analysis of ^{14}C-methyl incorporation and distribution of the radioactivity in methylated bases. Crude enzyme preparations from the hepatoma exhibited a 4-fold higher level of methylation than the corresponding preparation from normal liver. Subtle differences in the optimum pH of both enzyme systems occurred in the presence or absence of guanidine hydrochloride or guanidine carbonate. The effect of the latter was inhibitory. Further experiments suggested that the inhibitory effect of guanidine carbonate is due to the anion HCO_3^-. Methylation patterns were modified by varying pH values with the addition of 20 mM or 40 mM guanidine carbonate. The carbonate raised the relative content of methylated guanines as reflected by a decrease in other methylated components. The major products of the *in vitro* methylation were N^2-methylguanine and N^2-dimethylguanine. The latter was notably increased in the reaction products of the hepatoma enzyme, a finding that is consistent with previous studies on rat liver and kidney tumors and on human lymphocytes transformed by phytohemagglutinin. A notable feature of the methylation of normal liver enzyme was the formation of 2-*O*-methylribose; further study of this sugar methylation is warranted.

6478 RELATIONSHIP BETWEEN URINARY β-AMINOISOBUTYRIC ACID AND TRANSFER RNA TURNOVER IN CANCER PATIENTS.

(E.) Nielsen, H. R. (Sundby Hosp., Copenhagen, Denmark), K. Nyholm and K. E. Sjølin. *Cancer Res* 34(12):3428-3432, 1974.

The excretion of β-aminoisoburyric acid (B-AIB), a thymine catabolite, was investigated in 46 cancer patients. Twenty-four-hr urine samples were collected, and the β-AIB, 7-methylguanine (m^7G), and ψ-uridine isolated by thin-layer chromatography; the urinary urate was assayed using a uricase-UV method. In 68% of the patients studied, there was a positive correlation between the urinary excretion of β-AIB and that of ψ-uridine, while in 24% of the patients there was an increased excretion of ψ-uridine with a normal β-AIB excretion pattern. A genetic pattern involving increased urinary β-AIB and normal ψ-uridine excretion was seen in 13% of the patients; there was no elevation in m^7G excretion in these patients. Although in most cases the urate excretion rates were within normal limits, urate excretion often paralled that of ψ-uridine. Following resection of a colon tumor in one patient, there was a parallel increase in β-AIB and ψ-uridine on the second and third postoperative days and in ψ-uridine alone on the 68th postoperative day. The results indicate a positive correlation between urinary β-AIB and transfer RNA turnover in cancer patients.

6479 ANALYTIC TECHNIQUES IN THE SEPARATION AND IDENTIFICATION OF SPECIFIC PURINE AND PYRIMIDINE DEGRADATION PRODUCTS OF tRNA: APPLICATION TO URINE SAMPLES FROM CANCER PATIENTS.

(E.) Mrochek, J. E. (Oak Ridge Natl. Lab., Tenn.), S. R. Dinsmore and T. P. Waalkes. *J Natl Cancer Inst* 53(6):1553-1563, 1974.

An analytic technique for the quantification of RNA degradation products and its application to the determination of urinary excretion rates in normal and cancer patients was studied in order to determine if these waste products might be used to reflect disease status and therapeutic response in cancer patients. High resolution liquid chromatography was used to separate the purine and pyrimidine degradation products pseudouridine(psi-rd), 1-methyl-inosine(m^1I), N^2,N^2-dimethyl-guanosine(m_2^2G), 1-methyl guanosine (m^1G), and 7-methylguanine(7-MeGua) which were ultimately identified by gas chromatography and/or mass spectrometry. The mean amounts excreted in 24 hr by normal adults are as follows: 0.94 mg/kg psi-rd, 0.06 mg/kg m^1I, 0.06 mg/kg m_2^2G, 0.07 mg/kg m^1G, and 0.10 mg/kg MeGua. Normal children (4-13 years) excreted 60% more of the compounds than normal adults. Urinary excretion of psi-rd, m^1I, and m_2^2G were elevated in adult patients with neoplastic diseases. Results of a few analyses for m^1G and 7-MeGua genera-

ly seemed to parallel those of the other methylated
ompounds.

480 ON THE METHYLATED PURINE CONTENT OF tRNA PRESENT IN TUMOURS.

(E.) Ho, Y. (Univ.
ong Kong, Queen Mary Hosp. Compd.) and H. J. Lin.
r *J Cancer* 29(4):324-332, 1974.

ue to the increased methylase activities present in
arious tumors and transformed cells, a comparison
as made of the methylated purine content of tRNA
ulk extracted from human hepatomas with that in
ormal human liver to determine the association of
xcessive or aberrant methylation of nucleic acids
ith the process of carcinogenesis. Human primary
arcinomas of the liver were used as starting ma-
erial. A total of four tRNA specimens was analyzed.
ormal values were obtained from duplicate estima-
ions. Purine extimations in acid hydrolysates of
RNA were carried out by means of ion exchange chro-
atography on Dowe 50-H^+. The chromatographic pro-
iles of normal and tumor tRNA constituents were
imilar with respect to the number and positions of
he individual peaks. A comparison of normal tRNA
ith one of the specimens, a composite of several
reparations extracted from hepatomas, showed to be
ndistinguishable with respect to N^2-methylguanine
nd 1-methylguanine. All three tRNA specimens orig-
nating in cancer cases contained abnormally low
uantities of 7-methylguanine and of N^2-dimethyl-
uanine; whereas the quantity of 1-methyladenine was
omparable. This data did not support the hypothesis
hat tumor nucleic acids contain increased propor-
ions of methylated bases since the molar proportions
f the five bases detected (N^2-methylguanine, 1-
ethylguanine, 7-methylguanine, N^2-dimethylguanine,
nd 1-methyladenine) were not abnormally elevated.
he influence of possible contamination of specimens
as largely eliminated by comparison of their func-
ional activities and the results of the purine ana-
ysis are likely to be representative of normal and
epatoma tRNA.

481 A RAPID PROCEDURE FOR THE DETERMINATION OF DNA SYNTHESIS INHIBITION IN EHRLICH ASCITES MOR CELLS BY CYTOSTATICS.

(E.) Roussev, G. (Inst.
ochem. Bulgarian Acad. Sci.), E. Emanouilov, I.
gelov and E. Golovinski. *Proc Bulg Acad Sci* 27(12):
15-1717, 1974.

a new technique a "depot" of ^{3}H-thymidine is es-
blished in mice (Agnes-Blum strain) to increase
ailable time for testing cytostatics. Ascites
quid (0.3 ml) (30-40 x 10^6 cell/ml) of Ehrlich
perdiploid EAT strain was injected i.p. On the
venth day the cytostatics (hydroxyurea, actinomy-
ne D, mitomycin C, 6-mercaptopurine, 5-fluoruracil,
doxan, sarcolysine and ZBHL-51) were introduced
four i.p. injections at 2.5 hr intervals. One hr
ter the first injection each animal received i.p.
μCi of ^{3}H-thymidine. Nine hr later the animals
re sacrificed. The ascites cells were collected
d washed and the DNA concentration was measured
the two-wave length method. Radioactivity was
asured by scintillation spectrometry. The cells were treated with 0.3 N KOH for 18 hr at 37 C and then 1 N $HClO_4$ was added. After ten min in an ice bath the sediment was collected and treated with hot 1 N perchloric acid for 30 min. The percent inhibition of DNA synthesis was 97%, 79%, 91%, 33%, 36%, 55%, 76%, and 71% resp. for each of the cytostatics. Using this method the thymidine concentration remains constant for four to five days.

6482 INTERACTION OF dGMP RADICAL WITH CYSTEAMINE AND PROMETHAZINE AS POSSIBLE MODEL OF DNA REPAIR.

(E.) Willson, R. L. (Biochem. Dep., Brunel Univ., Middlesex, England), P. Wardman and K.-D. Asmus. *Nature* 252(5481):323-324, 1974.

On pulse radiolysis of nitrous oxide saturated solutions of thymidine, cytidine, adenosine, or guanosine (6×10^{-4} M, pH 4-5), transient absorption spectra attributable to the products of the reactions of the hydroxyl radical were observed. In most cases, no change in conductivity occurred, although with guanosine a large decrease in conductivity occurred immediately after the radiation pulse; such a change in conductivity in acid solution indicated the formation of positive ions. In similar studies with slightly acidic solutions of dGMP, radical cation formation was again detected as indicated by a change in conductivity immediately after the radiation pulse. Transient optical absorption spectra of oxidized guanosine and dGMP were similar in acid and neutral solution, indicating the presence of similar species. Additional support for the formation of radical-cations was provided by studies with dGMP using solutions containing excess bromide ions; the electrophilic radicals Br_2^-. were formed rapidly, after which they rapidly and exponentially decayed. On pulse radiolysis of nitrous oxide saturated solutions of 5' dGMP and promethazine, the radical-cation absorption was again observed initially but decayed rapidly and was replaced by new abosrption; a similar absorption was seen with solutions of guanosine but not with thymidine. The rate of formation of the new absorption increased with increasing promethazine concentration according to first order kinetics. Thus, the direct interaction between nucleophilic agents and free radicals produced in nucleic acid can occur rapidly. These processes constitute a 'repair' mechanism and could pertain to the repair of lesions induced by other electrophilic species such as those resulting from the biotransformation of carcinogenic or toxic agents.

6483 CELL CYCLE SYNTHESIS OF GLYCOLIPIDS INCLUDING THE FORSSMAN ANTIGEN.

(E.) Wolf, B. A. (Dep. Biol., Massachusetts Inst. Technol., Cambridge) and P. W. Robbins. *Cold Spring Harbor Conf Cell Proliferation* 1:473-479, 1974.

To determine the behavior of the Forssman antigen during the cell cycle synthesis of glycolipids, clone 2C of the NIL hamster line was isolated and used 6-10 passages after clonal isolation. These cells were synchronized with a double thymidine block and mitotic collection from monolayer with colcemide and labeled with (1-^{14}C)palmitate or (1-^{14}C)glucosamine-HCl. During palmitate uptake, GL-3 (Ceramide-Glu-Gal-Gal) and GL-4 (Ceramide-Glu-Gal-Gal-GalNAc) showed preferential labeling during G_1 and early S phases, while GL-5 (Ceramide-Glu-Gal-Gal-GalNAc-GalNAc) labeling was variant. GL-4 glucosamine incorporation was higher in G_1 and early S than in late S and G_2. GL-5, was labeled at all phases, but decreased in incorporation on late S and G_2. A complement fixation assay, highly specific and reacting only with GL-5, showed that the amount of antigen gradually increased 2-3-fold during the cell cycle. The binding of radioactive anti-Forssman antiserum to trypsinized cells increased gradually until eight hr after mitosis. No additional binding was observed when mitotic cells were trypsinized as the cell cycle progressed; the proportion of antigen exposed by trypsin treatment also increased until eight hr after mitosis. The two labels were used to reduce the possibility of inaccurate synthetic rates due to turnover of parts of the molecule; the equal rate of labeling of other glycolipids reduced the possibility of artifacts due to pool sizes. The author proposes three hypotheses as to the state of the cellular surface: (1) increasing reactivity due to a rounding of the cell is unlikely because G_1 cells expose a higher percentage of antigen than S cells, although both are completely flattened, (2) trypsin treatment or mitosis may result in greater mobility of membrane components, and (3) mitosis and G_1 may be the times in the cell cycle when the antigen sites are inserted into the membrane. The exposure of the Forssman antigen during mitosis as well as in the transformed cell indicates that the transformed cell surface may be locked in the "mitotic phase" of the cell cycle.

6484 DOPA DECARBOXYLASE IN MEDULLARY CARCINOMA OF THE THYROID.

(E.) Atkins, F. L. (Natl. Heart Lung Inst., Bethesda, Md.), M. A. Beaven and H. R. Keiser. *N Engl J Med* 289(11):545-548, 1973.

Thyroid tissues were obtained from 14 members of a family with a strong history of medullary thyroid carcinoma, pheochromocytoma, and parathyroid hyperplasia, and from one additional patient with a pheochromocytoma. Histaminase, decarboxylase, and tyrosine hydroxylase activities were assayed in the freshly excised tissues. Five patients had medullary thyroid carcinomas in which the activity of dopa decarboxylase was high compared with that in adjacent thyroid tissue or thyroid tissue from patients without the disease. Histaminase activity was also high in the tumor tissue, but histidine and ornithine decarboxylase could not be detected in the tumor or adjacent tissues. Tyrosine hydroxylase was found in the medullary thyroid carcinomas, being higher in two patients than in normal tissue. The activities of dopa decarboxylase and tyrosine hydroxylase were particularly high in the pheochromocytomas taken from 14 patients. Histaminase activity was low in all but two patients. The activity of dopa decarboxylase was inhibited by MK 486, α-methyldopa NSD-1055, and α-hydrazinohistidine in both medullary thyroid carcinoma and phenochromocytoma tissues; in neither tumor was the enzyme inhibited by α-methylhistidine. Mammalian dopa decarboxylase decarboxylated L-tryptophan and L-tyrosine, but to a lesser degree than L-dopa. The dopa decarboxylase activity in both tumors declined on storage at $^-$20 C, but was stable to freezing and thawing over short periods. The findings support the hypothesis of a defect in the neural ectoderm as the origin of the syndrome leading to the development of medullary thyroid carcinoma and pheochromocytoma.

6485 ABNORMAL GENE EXPRESSION ON PHENOTYPE OF SPECIAL PROTEASE IN HEPATOMAS.

(E.) Katunuma, N. (Sch. Med., Tokushima Univ., Japan) and Y. Banno. *Proc Int Symp Princess Takamatsu Cancer Res Fund, 4th, Tokyo* 137-148, 1973.

A proteolytic activity capable of inactivating several pyridoxal enzymes was detected in rat liver and was highly purified from the heavy mitochondrial fraction of the liver. The liver protease possesses many properties common to the group-specific protease of other organs but differs from group-specific proteases in other tissues in protein structure, based on molecular weight, electrophoretic patterns, immunoanalysis, and elution patterns on ion-exchange column chromatography. Regenerating liver and neonatal liver showed very low protease activity, suggesting a relation between the appearance of protease activity and the cell-division cycle. Varying activities of protease capable of inactivating ornithine transminase (OTA) were observed in all Morris hepatomas tested. Based on their physicochemical and enzyme chemical properties, the proteases in hepatomas with different growth rates appear to be identical with those in normal liver. The reprogramming of gene expression in some hepatomas is manifested by a marked rise in protease activity, but no organ-specific shift toward the muscle-type protein and enzymic properties was observed. Except for the most rapidly growing hepatomas, a reciprocal relation existed between OTA and protease activities. A double logarithmic plot of the two activities yields a straight line, suggesting that the level of OTA is controlled by the inactivating enzyme. In normal liver the level is not on the line or on an extension of the line. Thus, the intracellular OTA level may be controlled by additional regulatory mechanisms as well as by protease activity. The possible use of OTA-inactivating and other isoenzymes as marker enzymes for liver neoplasia is suggested; the enzymes may also have possible diagnostic use in liver biopsies or in plasma samples.

6486 PROPERTIES AND INTRACELLULAR LOCALIZATION OF EHRLICH ASCITES TUMOR CELL GLUTAMINASE.

(E.) Kovacevic, Z. (Med. Fac., Novi Sad, Yugoslavia). *Cancer Res* 34(12):3403-3407, 1974.

The intracellular localization of glutaminase in Ehrlich Ascites tumor cells was studied in order to clarify glutamine's role in oxidation metabolism of tumor cells. Ehrlich tumor cells maintained in Swiss mice were homogenized and fractionated using differential centrifugation. Ehrlich ascites tumor cells possessed phosphate-dependent glutaminase only. The Km for the enzyme was 4.5 mM, the pH optimum between 8.0 and 8.5, and the V_{max} 0.176 μM/min/mg protein. The glutaminase enzyme was found to be exclusively localized in the mitochondria, since it was invariably associated with glutamate dehydrogenase. Mitochondrial destruction resulted in the inactivation of the glutaminase enzyme, but borate protected the enzyme's activity when detergents were used to destroy the mitochondria. Digitonin treatment of the mitochondrial fraction revealed the glutaminase to be associated with the mitochondrial matrix. The role of glutaminase in energy metabolism of Ehrlich ascites tumor cells is shown to be significant due to its quantitative proportionality to growth rate and morphology of neoplasms.

6487 FURTHER STUDIES RELATING TO THE ROLE OF DEOXYRIBONUCLEASE IN CHROMOSOME BREAKAGE.

(E.) Paton, G. R. (Clin. Res. Cent., Harrow, England) and A. C. Allison. *Z Krebsforsch* 82(3):239-246, 1974.

The role of deoxyribonuclease (DNase) in chromosome breakage was studied in WI 38 human embryonic lung cells. Cell cultures were treated with pancreatic DNase (1200-2400 Kunitz units/mg) in hypertonic magnesium sulphate, fixed 20 hr later and examined for chromosome aberrations. Chromosome breakage was induced in 12-33% of dividing cells compared with a control value of 3%. The incidence of chromosome damage increased as the concentration of DNase increased, especially the number of chromatid breaks and exchanges in affected cells. It was suggested that the DNase attacked the chromosomal DNA and that the enzyme-induced chromosome damage occurred at some sensitive stage in the cell cycle. The DNase effect was unaltered by hydrocortisone treatment. Cells treated with 0.05% RNase showed that at least 9% of them had chromosome damage, but when hydrocortisone was included in the medium, the damaging effect of RNase was eliminated. The response of cells to treatment with 0.05% histone was similar to that with RNase, and the effect of the histone was eliminated in the presence of hydrocortisone. Thus it was suggested that DNase induced chromosome breakage by a lifferent mechanism than that for RNase and histone. 'he authors suggested that exogenous nucleases were ible to cross the cytoplasm and attack chromosomal 'NA in cultured human cells; and that in appropriate onditions endogenous lysosomal nucleases may also roduce a similar effect.

488 ALKALINE PHOSPHATASE ACTIVITY ASSOCIATED WITH THE NUCLEAR PORE IN NORMAL AND NEO-LASTIC SALIVARY GLAND TISSUE.

(E.) Cutler, L. S. Jniv. Connecticut Health Cent., Sch. Dent. Med., armington), A. P. Chaudhry and M. Montes. *J istochem Cytochem* 22(12):1113-1117, 1974.

uring the cytochemical investigation of human pa-
otid mixed tumors, focal alkaline phosphatase ac-
ivity was noted along the nuclear envelope of
ome of the neoplastic epithelial cells. To define
ne exact site of this activity along the nuclear
ivelope, benign mixed tumors of the parotid gland,
ormal parotid tissue, and normal parotid tissue from a patient who had a partial parotidectomy for a small cyst were incubated in media with and without substrate and examined by electron microscopy. About 20-30% of the neoplastic salivary gland epithelial cells displayed focal alkaline phosphatase reaction product deposits associated with their nuclear envelope. Similar products were occasionally seen in acinar and ductal cells of normal salivary glands and rarely seen in mesenchymal cells of tumorous and normal salivary tissue. In both normal and neoplastic epithelial cells, the product filled the nuclear pores without evidence of penetration into the nucleoplasm. The reaction appeared to be limited by the pore-associated material, with the reaction product assuming a geometric pattern. The pore size was about 761 Å, while the annulus diameter was about 1152 Å. All pores of a positive nucleus contained reaction product. Submandibular gland tissues from normal and atropine-treated rats showed similar nuclear pore site specificity in about 1-2% of their cells.

6489 PROGESTERONE METABOLISM IN ADENOCARCINOMA OF THE ENDOMETRIUM.

(E.) Collins, J. A. (Dep. Obstet. Gynecol., Univ. Western Ontario, Canada) and D. M. Jewkes. *Am J Obstet Gynecol* 120(6):779-784, 1974.

The metabolism of progesterone by homogenates of endometrial adenocarcinoma taken from 15 patients was studied. Progesterone-4-^{14}C was incubated with the homogenates and the resulting steroid products were isolated by thin-layer chromatography, radioassayed, and identified after recrystallization. The poorly differentiated adenocarcinomas (three of the tumor samples) appeared to produce the same five products as did proliferative and secretory endometrium and the well-differentiated adenocarcinomas. The steroids produced in largest quantities were 5α-pregnan-3,20-dione(5α-P) and 20α-OH-pregn-4-en-3-one (20α-OHP) (2.69 ± 0.24 and 1.4 ± 0.24 ng/100 mg/min, resp.). Homogenates of proliferative endometrium and well-differentiated adenocarcinomas produced 5α-P in significantly greater quantity than 20α-OHP, while the pattern was reversed in the homogenates of secretory endometrium. Compared with proliferative endometrium, the well-differentiated carcinomas converted more progesterone to metabolites, but the proportion converted to each metabolite was similar in the two tissues. The data suggest that 5α-reductase activity is dominant in glandular cells, and that 20α-OH-dehydrogenase activity is dominant in stromal cells.

6490 A MANYFOLD INCREASE IN SISTER CHROMATID EXCHANGES IN BLOOM'S SYNDROME LYMPHOCYTES.

(E.) Chaganti, R. S. K. (New York Blood Cent., N.Y.), S. Schonberg and J. German. *Proc Natl Acad Sci USA* 71(11):4508-4512, 1974.

Sister chromatid exchanges were investigated in lymphocytes from seven normal persons, three individuals with Bloom's syndrome affected homozygotes (genotype *bl*/*bl*), two unaffected carriers of the Bloom's syndrome gene (genotype *bl*/+), 1 Louis-Bar syndrome affected homozygote, two unaffected carriers of the Louis-Bar gene, two Fanconi's anemia affected homozygotes, and one unaffected carrier of the Fanconi's anemia gene. The cells were grown in bromodeoxyuridine and stained with 33258 Hoechst and Giemsa. While phytohemagglutinin-stimulated lymphocytes from normal controls had a mean of 6.9 sister chromatid exchanges/metaphase (range 1-14), those from persons with Bloom's syndrome had a mean of 89.0 (range 45-162). Frequencies of sister chromatid exchanges were normal in cells heterozygous for the Bloom's syndrome gene and in cells from homozygotes and heterozygotes of the Louis-Bar syndrome and Fanconi's anemia, two other rare disorders characterized by chromosome instability and predisposition to cancer. By following shifts in the pattern of staining from chromatid to chromatid, visual evidence was obtained that the quadriradial configurations characteristic of Bloom's syndrome represent exchanges between homologous chromosomes, apparently at homologous points. These results suggest that the increased exchange between nonsister-but-homologous chromatids and between sister chromatids in Bloom's syndrome are aspects of one and the same disturbance. Further study of this phenomenon in relation to the clinical features of Bloom's syndrome may contribute to the understanding of the biological significance of chromatid exchange in somatic cells.

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PREFACE

Carcinogenesis Abstracts is a publication of the National Cancer Institute. The journal serves as a vehicle through which current documentation of carcinogenesis research highlights are compiled, condensed, and disseminated on a regular basis. It represents an integral part of the Institute's program of fostering and supporting coordinated research into cancer etiology. Issues of *Carcinogenesis Abstracts* normally contain three-hundred abstracts and three-hundred citations (unaccompanied by corresponding abstracts). Abstracts and citations refer to the current scientific literature that describes the most significant carcinogenesis research carried on at the National Cancer Institute, other governmental agencies, and private institutions. *Carcinogenesis Abstracts* is intended to be a highly useful current awareness tool for scientists engaged in carcinogenesis research or related areas. The great number and diversity of publications relevant to carcinogenesis make imperative the availability of this service to investigators whose work requires that they keep abreast with current developments in the field.

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LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	It.	Italian
Ar.	Arabic	Jap.	Japanese
Bul.	Bulgarian	Kor.	Korean
Ch.	Chinese	Latv.	Latvian
Cz.	Czech	Lith.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
E.	English	Por.	Portuguese
Eston.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fl.	Flemish	Ser.	Serbo-Croatian
Fr.	French	Sl.	Slovak
Ger.	German	Sp.	Spanish
Gr.	Greek	Sw.	Swedish
Heb.	Hebrew	Th.	Thai
Hun.	Hungarian	Turk.	Turkish
Ic.	Icelandic	Uk.	Ukrainian
In.	Indonesian	Viet.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

ACTH	adrenocorticotropic hormone	mg	milligram(s)
ADP	adenosine diphosphate	min	minute(s)
AMP	adenosine monophosphate	ml	milliliter(s)
ATP	adenosine triphosphate	mm	millimeter(s)
C	degrees centigrade	MTD	maximum tolerated dose
cm	centimeter(s)	ng	nanogram (10^{-9})
CNS	central nervous system	pg	picogram (10^{-12})
cpm	counts per minute	p.o.	orally
DNA	deoxyribonucleic acid	ppm	parts per million
e.g.	for example	r	Roentgen
g	gram(s)	RBC	red blood cells (erythrocytes), red blood count
μg	microgram(s)		
hr	hour(s)	resp.	respectively
i.m.	intramuscular	Rev.	review (only in citations)
i.p.	intraperitoneal	RNA	ribonucleic acid
IU	international unit(s)	s.c.	subcutaneous
i.v.	intravenous	sec	second(s)
kg	kilogram(s)	U	unit(s)
LD_{50}	median lethal dose(s)	UV	ultraviolet
m	meter(s)	WBC	white blood cells (leukocytes), white blood count
M	molar		
mEq	milliequivalent(s)	wk	week(s)
mM	millimolar	wt	weight(s)
μM	micromolar	yr	year(s)
mC,μC	milli-,microcurie(s)		

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6601 CARCINOGENESIS *IN VITRO*. *(E.)* Ponten, J. (No affiliation). *Recent Results Cancer Res* 44:98-102, 1974.

Three operationally distinct forms of *in vitro* malignant transformations have been defined: irregular growth transformation; unrestrained growth transformation; and infinite growth transformation. Synonomous with these types of alteration are, respectively, lack of the contact inhibition of locomotion, lack of the density-dependent inhibition of cell multiplication, and establishment of a permanent cell line. Normal cells show none of these transformations, their cell locomotion being restricted by contact with other cells. Similarly, cell proliferation is normally under strict control; as cell density increases and confluence is reached, the multiplication rate drops until it becomes virtually zero. A third type of control is cellular aging, which prevents normal cells from undergoing an infinite number of divisions. Many agents induce cell transformation *in vitro*, and most are also oncogenic *in vivo*. Serial cultivation *per se* causes transformation in cells from unstable species (e.g., mouse, rat, hamster), but not in stable species (human, chicken). With unstable species, there are no principal differences between cells transformed *in vitro* and cells from cancers already established *in vivo*. However, differences in behavior are apparent when stable species are considered. One point of view holds that cell transformation *in vitro* is entirely irrelevant to the understanding of spontaneous cancers, while another view asserts that viruses and carcinogens effective *in vitro* should be looked upon as valuable experimental tools rather than putative natural human carcinogens. For example, feline sarcoma virus and SV40 cause a much more irregular and unrestrained growth transformation in glia cells than is found in spontaneously induced glioma cells. Determination of the reason for this difference must be the aim of further investigation. (No references)

6602 RIBOSOMES AND CANCER. *(E.)* Delaunay, J. (Inst. Mol. Pathol., Paris, France) and G. Schapira. *Biomedicine* 20(5):327-332, 1974.

Due to the role played by ribosomes in the expression of genetic information, ribosomal structure and function in healthy and carcinoid states were reviewed. The nature of ribosomal binding to the endoplasmic reticulum may be altered in cancer, as indicated by differences in polysome size in healthy and cancerous cells. Ribosomal rRNA may also be different in cancerous cells by containing either more or fewer nucleotides than normal. Ribosomal protein analysis by polyacrylamide gel electrophoresis has shown differences between healthy and cancerous tissues. Actinomycin D and camptothecin are two drugs which, in low concentrations, may be used to block nucleolar synthesis of 45 S RNA selectively, and are thus useful in investigations of rRNA's role in cancer. The vinca alkaloids are also useful due to their ability to inhibit 18 S RNA synthesis in human cells. Ricin and arbrin, which inhibit tumor growth, and aflatoxin B$_1$, which is a carcinogen, are also useful agents due to their abilities to alter RNA synthesis in different ways. It is concluded that there is difficulty in establishing causality in the relationship of ribosomes and cancer. (51 references)

6603 CYCLIC ADENOSINE MONOPHOSPHATE AND PROSTAGLANDINS IN ONCOLOGY. *(Rus.)* Bershtein, L. M. (N. N. Petrov Sci. Res. Inst. Oncol., Leningrad, USSR) and V. M. Dil'man. *Vopr Onkol* 20(3):105-120, 1974.

It has been demonstrated that malignant transformation is associated with a cyclic adenosine monophosphate (cAMP) deficiency in murine fibroblasts and in a number of virus-induced tumors in experimental animals. Prostaglandin (PG) E$_1$ increases cAMP contents of both normal and transformed fibroblasts. Both PG and cAMP inhibit growth of transformed cells and increase differentiation of various tumors cultured *in vitro*. The decrease in the cAMP content of cells undergoing malignant transformation is apparently due to both inhibition of adenyl cyclase and changes in the response of the enzyme to hormone stimulation. Some investigators have found that urinary cAMP excretion is decreased in human cancer patients and tumor-bearing animals, but these results have not been confirmed. PG is secreted in large quantities by a variety of human tumors and accounts for the development of "ectopic endocrine syndromes" in some cases. Administration of indomethacin and aspirin, which block biosynthesis of PG, reduces the content of PG in tumors and reduces the tumor size in tumor-bearing animals. The growth of some animal tumors has been inhibited by cAMP, its derivatives, and theophylline. Synthetic polynucleotides act by activating cAMP formation and by increasing the immune response. The chemical structure, biosynthesis, and metabolism of cAMP and PG, their biological effects and possible action mechanisms, and their effects on some aspects of cell viability are also reviewed. (159 references)

6604 ROLE OF CYCLIC AMP IN THE CONTROL OF EPIDERMAL CELL GROWTH AND DIFFERENTIATION. *(E.)* Voorhees, J. J. (Univ. Michigan Med. Sch., Ann Arbor), E. A. Duell, L. J. Bass and E. R. Harrell. *Natl Cancer Inst Monogr* 38:47-59, 1973.

A model of epidermal growth control is presented which hypothesizes the cyclic AMP system as the major regulatory mechanism in epidermal proliferation and differentiation. This hypothesis is an attempt to interrelate the original epinephrine-chalone mechanism, the presence of glycogen in regenerating epidermis, the evidence implicating cyclic AMP in the control of growth in various tissues and cells in culture, Crick's morphogenetic gradient hypothesis, and the authors' observations on the cyclic AMP system in normal mammalian epidermis and in the rapidly proliferating epidermis of psoriasis. In this model, any injury, no matter how minor, would perturb the spatial architecture or the molecular constituents of the epidermal cell surface, thus decreasing adenyl cyclase activity and, in turn, cyclic AMP levels. Such a decrease in cyclic

AMP would then switch the synthetic processes characteristic of normal epidermal differentiation to a program of cell replication with its concomitant accumulation of glycogen. As soon as the newly formed cells entered G_1 phase, some of them would differentiate and produce normal levels of cyclic AMP and consequently normal glycogen levels. In this scheme, even very local or superficial damage could trigger proliferation in the basal cells. The intracellular levels of cyclic AMP may not be under primary hormonal control but may be controlled by a specific local substance previously called a "chalone". It is claimed that the notion of examining the ability of cell-surface molecules to enhance epidermal differentiation is new to the chalone concept and requires the examination of the response of biochemical markers of epidermal differentiation to chalone candidates. (138 references)

6605 PROTEIN MODIFICATION, METABOLIC CONTROLS, AND THEIR SIGNIFICANCE IN TRANSFORMATION IN EUKARYOTIC CELLS.

(E.) McCarty, K. S. (Duke Univ. Med. Cent., Durham, N.C.) and K. S. McCarty, Jr. *J Natl Cancer Inst* 53(5):1509-1514, 1974.

Postsynthetic modification of proteins appear crucial in metabolic control, transcription, and differentiation. The modifications considered are phosphorylation, methylation, acetylation, adenylylation, and glycosylation. Phosphorylation has the capacity to regulate enzymes, while phosphorylation, methylation, and acetylation all have the potential to alter both transcription and replication of DNA by way of their effects on chromosome proteins. Phosphorylation may either stimulate or repress enzyme activity. For example, phosphorylation of glycogen phosphorylase *b* converts this enzyme to the more active glycogen phosphorylase *a* whereas the phosphorylation of glycogen synthetase *a* converts it to the less active glycogen synthetase *b*. Possible direct regulation of transcription by postsynthetic phosphorylation was suggested by the phosphorylation of the DNA-dependent RNA polymerase σ factor from *E. coli* with a protein kinase from rabbit muscle. These and other phosphorylase reactions utilize ATP as a substrate and are performed by protein kinases, many of which are cyclic AMP (cAMP)-dependent. Possible influence of methylation was suggested by the observation of the presence of methylarginine in the nonhistone acidic chromosome proteins in rat liver and of methylated lysine in ribosome proteins of *Blastocladiella emersonii*. As to acetylation, amino terminal acetyl, which is associated with some proteins at their synthesis, appears metabolically stable, in contrast to internal acetyl, which is metabolically less stable and represents a postsynthetic modification. Further, internal acetylation of lysine residues in some histones has been found. Capacity for postsynthetic modification in the control of DNA synthesis is seen in the transfer of the adenosine diphosphoribose moiety of NAD to nuclear proteins along with the liberation of nicotinamide. Enzyme adenylylation has the potential of transcriptional control as shown by the finding that DNA-dependent RNA polymerase of *E. coli* is reversibly inactivated by an adenylylation type of mechanism. Glycosylation further exemplifies postsynthetic modification which can alter cell metabolism. For example, glycosylation of secreted proteins occurs when the protein, which is synthesized in the rough endoplasmic reticulum, passes through the Golgi apparatus before it is released from the cell or becomes part of the cell membrane structure. Malignant transformation may be related to postmodification of the many proteins involved in carbohydrate metabolism. Evidence may reside in certain observed effects of cAMP, such as changing the cell morphology, growth rate, and density of transformed cells towards corresponding properties of normal cells. Since many protein kinases appear to be activated by cAMP, these effects of cAMP suggest that alteration of regulation of enzyme activity (i.e., alteration in postsynthetic modification of enzyme proteins) occurs during transformation. (59 references)

6606 ERYTHROPOIETIN AS A NONSPECIFIC GROWTH FACTOR AND ITS EFFECT ON CARCINOGENESIS.

(Rus.) Finogenova, M. A. (Inst. Nutr., Moscow, USSR). *Vopr Onkol* 20(5):93-97, 1974.

Studies are reviewed of the action of erythropoietin as a nonspecific growth factor stimulating cell division and tumor growth *in vivo* and *in vitro*. The first experiments showed tumor stimulation in rats with Novikov hepatoma by injecting erythropoietin 24-48 hr prior to tumor implantation. Later, erythropoietin stimulation was observed *in vitro* in the culture of bone marrow cells from the patient with monocytic leukemia. Controvertible results were obtained in *in vivo* studies of the effect of erythropoietin on leukemia. In BALB/c mice injected s.c. and i.p. with 0.05 ml of leukemia spleen extract and 0.5 ml of erythropoietin for eight weeks, leukemia developed in 11% of the experimental animals and 52% of the controls. In CFW female mice, however, erythropoietin increased the incidence of leukemia. Original retrospective studies showed a stimulating effect of erythropoietin on mice with skin tumors induced by 20-methylcholanthrene in conventional (0.5-1 ml of rat erythropoietic serum injected i.p.) and cocarcinogenic (exposure to cobalt or 0.5 atm pressure) conditions. Further research demonstrated that in addition to stimulating erythropoiesis, erythropoietin also acts as a tumor growth factor. Experiments and clinical observations demonstrated erythropoietin involvement in polycythemia developing against the background of malignant tumors of various localization. It is concluded that in relation to malignant cells, erythropoietin acts as a nonspecific growth factor both *in vivo* and *in vitro*. (42 references)

6607 INDUCTION AND SOME CHARACTERISTICS OF "MINIMAL DEVIATION" AND OTHER TRANSPLANTABLE RAT HEPATOMAS.

(E.) Morris, H. P. (No affiliation) and D. R. Meranze. *Recent Results Cancer Res* 44:103-114, 1974.

Over a 20-yr period a spectrum of hepatomas and other transplantable tumors was induced in inbred rat strains by the feeding of chemical carcinogens. The tumors are classified according to inducing agent and levels, growth rate, histologic type, metastases, chromosome number, and the sex of the host. Tumors showing different degrees of differentiation are separated into three groups according to their degree of differentiation as described by many morphologists. Glucose metabolism and thymidine utilization in poorly differentiated, rapidly growing tumors and in well-differentiated, slow growing tumors are contrasted. Except for loss of feedback control in cholesterol synthesis, the hepatomas show a wide diversity of metabolic patterns. Some of the key enzymes of carbohydrate and nucleic acid metabolism appear to be correlated with growth rate. The most minimal hepatomas have a normal karyotype. In other hepatomas with aneuploid chromosome numbers, glycogen and four enzymes of carbohydrate metabolism are absent or very low. (22 references)

6608 EXCISION REPAIR OF DNA BASE DAMAGE. *(E.)* Cerutti, P. A. (J. Hillis Miller Health Cent., Univ. Florida, Gainsville). *Life Sci* 15(9): 1567-1575, 1974.

The excision repair pathway of DNA repair is reviewed. The first step in this process is recognition of the damaged portion of the DNA molecule by a "repair" endonuclease which then excises the damaged strand at the proximal end of the lesion. Endonuclease activity has been found in eukaryotic cells and several repair endonucleases have been described in bacteria; 1) endonuclease II which recognizes apurinic sites and can initiate repair of alkylating agent-induced lesions; 2) an endonuclease which recognizes exclusively apurinic and probably apyrimidinic sites; 3) endonucleases incising x-ray damaged DNA; and 4) endonucleases which specifically incise in close proximity on the 5-side of cyclobutane-type photodimers ("UV-endonucleases"). Experimental evidence indicates that 7-alkylguanine, formed by most alkylating agents, and 5-hydroxymethyl- or 5-hydroxyperoxymethyl-uracil, formed by ionizing radiation, are not removed by endonucleases. The second step in excision repair is exonucleolytic degradation of the damaged DNA strand. In bacterial systems, long DNA fragments (> 1500 nucleotides) are degraded by exonuclease V along with the exonucleolytic action of polymerases II and III while short strand degradation (10-30 nucleotides) is effected by the exonuclease associated with polymerase I. All 3 polymerases seem to take part in rebuilding the DNA strand. The final step in the repair process is sealing of the phosphodiester bond between the newly synthesized portion of DNA and the preexisting DNA strand. This is done by polynucleotide ligase, an enzyme which has been purified from mammalian tissue. (75 references)

6609 MINIREVIEW. REPAIR OF DNA IN MAMMALIAN CELLS. *(E.)* Strauss, B. S. (Dep. Microbiol., Univ. Chicago, Ill.). *Life Sci* 15(10):1685-1693, 1974.

DNA lesions, bacterial repair, excision repair in mammalian cells, and post replication repair are reviewed and the relationship between repair and carcinogenesis is briefly discussed. One class of lesions is produced by UV and agents with similar effects and a second class is produced by agents with effects similar to X- or gamma radiation. Three bacterial repair mechanisms are known: one depends on a photoreactivation (PR) enzyme which uses the energy of visible light to split carbon-carbon cyclobutane bonds thereby releasing the monomers. The ability of PR enzyme and light to reverse a biological effect is taken as evidence that the significant lesion is a pyrimidine dimer. The second mechanism, excision repair, involves a preliminary nucleolytic cut in a DNA molecule containing a lesion, the exonucleolytic removal of the sequence including the damaged nucleotides, and a resynthesis of the excised region using the remaining undamaged strand as a template. Post replication repair, the third mechanism, involves DNA synthesis from a template containing lesions. DNA synthesis halts at or near the site of the lesion and reinitiates at a normal point. The resulting gap is later filled by a recombination event. Excision repair in mammalian cells has been observed by a number of methods, and includes repair of lesions induced by X-rays, UV, and a variety of nucleophilic reagents. Cells from patients suffering from certain forms of the genetic disease Xeroderma pigmentosum are unable to excise dimers from their DNA and therefore do not carry out excision repair at all. Such cells are nonetheless able to synthesize DNA after small doses of UV by the post replication repair mechanism. Those patients who are unable to carry out excision repair are more likely to develop skin cancer. If the repair mechanisms play some biological role, this observation suggests that there is an inverse relationship between repair and carcinogenesis. It seems reasonable to suppose that errors do occur when DNA synthesis proceeds while lesions remain in the DNA, for example in Xeroderma cells containing pyrimidine dimers. It is suggested that cancer is the result of either post replication repair or the "normal" synthesis that occurs while lesions are still in the DNA. It is concluded that excision repair is a biological protective device which avoids replicating DNA containing lesions. (40 references)

6610 CHEMICAL AND BIOCHEMICAL FACTORS THAT CONTROL THE TOXICITY AND CARCINOGENICITY OF ARENE OXIDES. *(E.)* Jerina, D. M. (Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, Md.). *Biochem Dis* 4 (Part A):289-308, 1974.

The factors that control the enzymatic formation and disposition of arene oxides as well as the propensity of these compounds to isomerize to phenols and react with nucleophiles are discussed. There is direct evidence for the formation of an arene oxide in a metabolic system. This was achieved by incubating $[^{14}C]$ naphthalene with the liver microsomal system in the presence of added naphthalene oxide. Isolation of the carrier napthalene oxide and purification to remove the radioactive naphthalene, naphthol, and dihydrodiol showed that radioactivity was incorporated into the carrier as naphthalene oxide. Arene oxides have been implicated as potent carcinogens in cell culture systems, as frame-shift mutagens, and as causative agents in tissue necrosis. The kinetics and mechanism of isomerization of a number of arene oxides have been studied in detail. The mechanisms that have been discovered are those that cause the intramolecular migration and retention of substituents during the course of aryl hydroxylation ("NIH shift"). Stability of an arene oxide toward rearrangement to the corresponding phenol is a critical factor in determining its biological activity. Electron donating groups facilitate the isomerization and decrease the lifetime of an arene oxide, while electron withdrawing groups increase its lifetime. Two other major classes of reactions which occur with arene oxides under physiologic conditions are hydration to dihydrodiols by epoxide hydrase and enzymatic as well as nonenzymatic addition of glutathione. Both of these reactions convert the arene oxides into compounds that appear to be innocuous to the organism. After the initial enzymatic formation of an arene oxide, chemical rearrangement to a new arene oxide is possible such as with naphthalene and pyrene which rearrange to phenols. Studies on bromobenzene-induced necrosis show that bromobenzene can exert enzymatic control of levels and biologic activity of arene oxides. Induction and inhibition experiments link bromobenzene-induced necrosis to metabolic activation of the bromobenzene. Differences in the ability of substrates such as benzene, toluene or xylene to induce necrosis seem best related to the stability of the possible intermediate arene oxides toward rearrangement to phenols. (40 references)

6611 FORMATION OF CARCINOGENS FROM PRECURSORS OCCURRING IN THE ENVIRONMENT: NEW ASPECTS OF NITROSAMINE-INDUCED TUMORGENESIS. *(E.)* Preussmann, R. (No affiliation). *Recent Results Cancer Res* 44:9-15, 1974.

The experimental evidence for the formation of carcinogenic N-nitroso compounds from appropriate precursors is reviewed, and the consequences of such reactions are discussed. The acid milieu of the stomach provides particularly favorable conditions for nitrosation of secondary amines and amides; in the presence of certain enterobacteria, nitrosamine synthesis can even take place at a neutral pH and when nitrate is used. Simultaneous p.o. administration of secondary amines or amides and nitrate to experimental animals induced tumors at the same sites as those induced with the corresponding N-nitroso compound. One outstanding characteristic of N-nitroso compounds is their capacity for inducing tumors in practically any important organ in mammals (e.g., liver, lung, stomach, esophagus, brain and nervous system, kidney, and bladder). The organ selected can be dependent on the structure, dosage, or mode of administration of the compound, or on the species. By treating pregnant rats with ethyl urea and nitrite during the second half of pregnancy, tumors were induced in the brain and nervous system of offspring. These tumors were typical of those induced by ethylnitrosourea, suggesting that ethylnitrosourea had been produced *in vivo* from the ethylurea and nitrite administered. (34 references)

6612 THE SPECTRUM OF ASBESTOS-RELATED DISEASES. *(E.)* Rom, W. N. (Sacramento Med. Cent., Calif.) and P. E. S. Palmer. *West J Med* 121(1):10-21, 1974.

Asbestos as a cause of environmentally-related illnesses is discussed with regard to the characteristics of the mineral and the spectrum of diseases produced by exposure to it. The major risk related to exposure to asbestos dust is that of pulmonary fibrosis, a risk which is increased many times if the patient is also a smoker. However, exposure to asbestos also increases the risk of carcinoma of the lung, again, especially if the patients is a smoker. It seems probable that exposure to asbestos, particularly in small amounts, increases the likelihood of a mesothelioma developing, although a close relationship to the degree of exposure is not firmly established and it is unknown when mesotheliomas develop in some patients while others develop carcinomas of the lung. In some geographical areas exposure to asbestos carries an increased risk of malignant disease of the alimentary tract but it is not certain that this risk is present in every locality. It is unlikely that asbestos by itself is the sole cause of malignant disease, and it is possible that some other factor(s) working in association with it will be shown to cause the presently inexplicable variation in the malignant diseases occurring among asbestos workers. No consistent common denominator has yet been associated with asbestos exposure nor has the subsequent development of disease been defined. (73 references)

6613 CIGARETTE LIGHTING AND LUNG CANCER: A NEW PERSPECTIVE. *(E.)* Marienfeld, C. J. (Dep. Community Health Med. Pract., Univ. Missouri, Columbia). *Perspect Biol Med* 18(1):44-56, 1974.

The role of cigarette lighting in relation to lung cancer is reviewed. Soot-condensation experiments demonstrated that unburned carbon soot emanates from lighted paper book-type wax-impegnated matches and from burning wick-type cigarette lighters. Benz(a)pyrene-related compounds were absorbed upon the soot which is produced when book-type paper matches and cigarette lighters were used to light smoking tobacco. Chromium compounds (which have been shown to be carcinogenic) were present in match heads examined by atomic absorption spectrophotometry and are probably volatilized upon lighting. It is suggested that the repeated inhalation of such carcinogenic materials may be contributing to the increasing lung cancer incidence occurring particularly among cigarette smokers. This factor, when added to the carcinogenic potential of tobacco and urban and industrial air pollution, may explain several apparent epidemiologic inconsistencies. (25 references)

6614 TRACE METALS IN MAN'S ENVIRONMENT AND THEIR DETERMINATION BY ATOMIC ABSORPTION SPECTROSCOPY. *(E.)* Norval, E. (Natl. Phys. Res. Lab., CSIR, Pretoria, South Africa) and L. R. P. Butler. *S Afr Med J* 48(63):2617-2626, 1974.

The clinical effects, sources, and techniques for atomic absorption spectroscopy determination of metals in air, water, and biological samples are reviewed. Atomic absorption spectroscopy is a low cost technique for rapid, accurate determination of very small concentrations of metals in the environment. Lead, mercury and cadmium, the chief sources of dangerous environmental pollution, are discussed with regard to their physiological significance and determination methods. The roles of lead, iron, chromium, manganese, vanadium, nickel, zinc, arsenic and beryllium in the etiology of occupational diseases are also discussed. It is concluded that atomic absorption spectroscopy is a useful method for defining the precise functions of metals in metabolism, diagnosing pathological conditions and defining maximum allowable environmental concentrations of trace and macro metal contaminants. (129 references)

6615 NATURALLY OCCURRING MUTAGENS OF BIOLOGICAL ORIGIN. A REVIEW. *(E.)* Tazima, Y. (Natl. Inst. Genet., Mishima, Japan). *Mutat Res* 26(4):225-234, 1974.

Naturally occurring mutagens were reviewed. Pyrrolizidine and some other alkaloids contained in higher plants (*Senecio, Crotararia, Heliotropium*), cycasin in cycads, bracken toxins in ferns and several kinds of mycotoxins produced by fungi were covered. Chemical structures and major biological effects of these substances are discussed in relation to their carcinogenicity and mutagenicity. Cycasin and sinigrin are not mutagenic by themselves, but their metabolites are known to be mutagenic. From the point of view of genetic hygiene, the most important group may be the mycotoxins, because these are contaminants of several sources of human food stuffs. The aflatoxins, one group of these substances, are the most active liver carcinogens and were discovered as the result of outbreaks of toxicosis in poultry, cattle, pigs, and sheep due to eating ground nut. The antibiotics, another group of mycotoxins, are patulin and penicillic acid. The carcinogenic property of both substances was revealed following repeated s.c. injection in the rat. No evident carcinogenicity, however, has been observed yet in animals following ingestion. Mutagenic investigations of the mycotoxins are as yet very limited, but increasing evidence for a close relationship between carcinogenicity and mutagenicity suggests that most of them are mutagenic. In view of the probable significance of mycotoxins on the incidence of spontaneous mutations as well as etiology of cancer, the necessity for further investigation is stressed. (51 references)

6616 ONCOGENIC VIRUSES IN PRIMATES: A REVIEW. *(E.)* Deinhardt, F. (Rush-Presbyterian-St. Luke's Med. Cent., Chicago, Ill.) and J. B. Deinhardt. *Rush-Presbyt St Luke's Med Bull* 13(2):67-79, 1974.

The current status of viral oncogeny in primates is reviewed. The basic host-virus interrelationships have been established for several tumors induced in nonhuman primates with C-type RNA and herpes-like DNA viruses. Nonhuman primates are ideal subjects for investigation of the pathogenicity of C-type and mouse mammary tumor virus (MMTV)-like viruses, which are endogenous in man and other primates. Antigenic and nucleic acid differences between true oncogenic and endogenous viruses and their similarities to viruses from other species are poorly understood. The oncogenicity of a human virus in primates has been established by *herpesvirus ateles* (HVA)- and *herpesvirus saimiri*- induced lymphomas and leukemias and by Epstein-Barr virus (EBV)-induced tumors; such tumor induction supports the suggestion that EBV causes some human neoplasms. Other possible pathogenic mechanisms include C-type RNA virus activation of EBV, EBV activation by C-type RNA virus-transformed cells, and EBV-C-type RNA virus interaction. The recent transformation of cultured primate cells (including human) by HVA may enable transformation of lymphoblastoid cell lines derived from isolated B- or T-cell clones, incidentally providing an important immunologic tool. Viral oncogeny in nonhuman primates has important implications for the study and treatment of human neoplasms. (142 references)

6617 EXPERIMENTAL EVIDENCE FOR THE ROLE OF VIRUSES IN HUMAN CANCER. *(E.)* Zur Hausen, H. (Univ. Erlangen-Nurnberg, West Germany) and H. Wolf. *Proc Fifth Int Symp Biol Characterization Hum Tumors* 1:80-84, 1974.

Various laboratory studies are reviewed which point to an etiologic relationship between Epstein Barr virus (EBV) and Burkitt's lymphoma and nasopharyngeal carcinoma. Evidence was obtained by annealing Burkitt tumor cell RNA with EBV DNA, hydridization of Burkitt lymphoma and nasopharyngeal carcinoma biopsy DNA with EBV DNA, and visualization of nuclear localization of virus-specific complement-fixing antigens using anti-complementary immunofluorescence. Epithelial cells in the nasopharyngeal carcinoma were shown to harbor EBV genomes. The relationship of herpes simplex virus type II to human cervical cancer is less clear, since hybridization could not be demonstrated between tumor biopsy DNA and the virus DNA. The relationship of a hypothetical mammary tumor virus to human mammary cancer is suggested by successful hybridization of human mammary tumor RNA with mouse mammary tumor virus DNA. Similarly, RNA from human leukemias, sarcomas, and lymphomas anneal with DNA transcribed from Rauscher mouse leukemia virus RNA. (25 references)

6618 ENDOGENOUS VIRUSES IN NORMAL AND TRANSFORMED CELLS. *(E.)* Todaro, G. J. (Natl. Cancer Inst., Bethesda, Md.). In: *Developmental Aspects of Carcinogenesis and Immunity* edited by T. J. King (New York: Academic Press, Inc., 1974) pp. 145-158.

The existence and possible role of naturally occurring endogenous type C viruses in normal and transformed cells is reviewed. Endogenous, genetically transmitted type C viruses are represented in the DNA of somatic cells of a great many species in a repressed form. The viruses produced by both normal tissues and tumorigenic tissues are similar in their morphological, biochemical, and immunological properties. All of them have reverse transcriptase, an enzyme within the virus particle capable of transcribing viral RNA back into DNA. Type C viruses can be detected by electron microscopy, biological activity (focus formation, or rescue of nonproducer virus), immunodiffusion, complement fixation, radioimmunoassay of interspecies group-specific antigens, recognition of virus-specific reverse transcriptase by immunoadsorbant column or polymerase antibody and RNA-DNA hybridization. There are now at least seven species (chicken, Chinese hamster, Syrian hamster, mouse, rat, cat, and pig) in which it has been clearly shown that a complete copy of the endogenous type C virus, the virogene, is present in the normal cells. Properties of endogeneous viruses include their inability to infect cells that produce them, their presence in many (perhaps all) species, and their greater likelihood to be produced by transformed rather than by normal cells. The transformed cells that produce virus appear to be less tumorigenic than the transformed cells that do not produce virus. This suggests that such a system might have a protective role; virus expression might protect on an immunologic basis against tumor formation. (45 references)

6619 HOST-VIRUS RELATIONSHIP AT THE EMBRYONIC LEVEL.

(E.) Solter, D. (Wistar Institute, Philadelphia, Pa.), W. Biczysko and H. Koprowski. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 3-30.

The ease with which mammalian embryos can be maintained in tissue culture during the first half of gestation made possible the study of cellular events associated with the presence of endogenous virus and of the response of embryonic cells and tissues to infection with exogenous oncogenic particles. Results of such studies indicate that type A virus particles become visible in large concentrations following the first mitotic division of the egg, after fertilization or after parthenogenetic stimulation. The concentration of type A particles in embryonic cells decreased markedly at the blastocyst state, when type C particles are first seen by electron microscopy. The granular, heterogenous nucleolar RNA may be shown to be the site of synthesis of type A particles, particularly if it can be demonstrated that 30-40S RNA is a precursor of 70S RNA found in mature type A particles in nonembryonic cells. The response of embryonic cells to infection with the DNA oncogenic viruses indicates that behavior of such viruses in undifferentiated cells and in cells at various stages of differentiation varies from that observed in fully differentiated somatic cells. Permissiveness of mouse embryonic cells for replication of SV40 and resistance of 2-cell mouse embryos to attachment of polyoma virus are examples of such differences. Infection of blastocysts and egg cylinders with both polyoma virus and SV40 may indicate that viruses for which adult cells are not permissive could, at the embryonic level, participate in the process of teratogenesis or even oncogenesis. Although the study of endogenous viruses in the embryo and the interaction of early embryonic cells with exogenous virus is still in its preliminary stages, this model may play an important role in exploring the mechanisms involved in differentiation and oncogenesis. (91 references)

6620 THE CELL SURFACE, VIRUS MODIFICATION, AND VIRUS TRANSFORMATION.

(E.) Sheinin, R. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 371-400.

Virus-cell interactions in which host cells survive, but with virus-determined biochemical alteration of the cell surface, are especially important in assessing the potential for oncogenic conversion by viruses. The cell surface embodies a key lesion of neoplastic transformation since it mediates the phenomena of cell-cell interaction that underlie both normal and cancerous growth and development. Genetic considerations suggest that the oncogenic virus genome coding for transformation does so through interaction with cell genetic information that determines structure and function of surface molecules. The changes which are observed on the surfaces of virus-transformed cells are extensive and may be described both in functional (enzyme activity, transport system, virus interaction, agglutinin interaction, antigenicity, immune system interaction, contact inhibition of growth, morphogenesis, and interaction with regulatory molecules) and biochemical (glycoproteins at periphery of plasma membrane, glycoproteins within plasma membrane, glycolipids, and sialic acid residues) terms. These changes are difficult to explain with the available genetic repository. An accomodation may come from a model which assumes that integrated virus genome interferes with cellular regulatory processes that normally control the formation and turnover of surface molecules. Such a model would be based on the following observations: (1) virus transformation is associated with the appearance of species-, tissue-, or even cell-specific surface alterations not mediated by the virus genome; (2) cells that undergo transformation suffer a derepression of certain pathways of surface molecule synthesis; (3) virus-transformed cells do not exhibit a shift in metabolic activity during the growth cycle; (4) cell surface formation in virus-transformed cells mimics in some respects cells which have not completed their full cycle of duplication; (5) increased activity of plasma membrane-associated proteases in transformed cells; and (6) pleiotypic surface alterations exhibited by virus-transformed cells are evoked in normal cells by a number of agents. (175 references)

6621 REPLICATION AND INTEGRATION OF THE GENOME OF ONCORNAVIRUSES.

(E.) Vigier, P. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974), pp. 209-233.

The structure and replication of the viral genome in permissive and nonpermissive cells is discussed, with special attention to data which suggest that the viral genome or the DNA "provirus" is integrated with the cellular genome. The genome of oncornaviruses consists of high molecular weight RNA with a sedimentation constant of 60S-70S; virions contain RNA's of lower molecular weight. Sarcoma viruses must accomodate the information that controls transformation; it has been reported that the Rous sarcoma virus (RSV) can contain 23 proteins. If all these proteins are coded for by the virus, at least two subunits would be necessary to accommodate all the information. Cells infected with avian and murine viruses contain DNA that hybridizes with the viral 60S-70S RNA. Reverse transcriptases from oncornaviruses of different animal species differ antigenically and can be characterized immunologically; nonpermissive cells transformed by oncornaviruses may not contain any detectable amount of reverse transcriptase. Probably all of the genetic information of avian oncornaviruses can be transmitted by DNA associated with that of virus-producing or nonproducing transformed cells. Induction of virus able to infect pheasant cells and help in the production of infectious RSV(0) was observed after treatment of certain group-specific (gs^+) lines with X-rays, UV light, mitomycin D, urethane, 20-methylcholanthrene, and 4-nitroquinoline-1-oxide. Gs^- cells also carry a cryptic virus; the chromosomal locus that controls the presence of natural gs antigen in chicken cells does not represent the viral genome itself but regulates its expression. Genetic determinants of murine leukemia viruses are present in all strains of mice. (116 references)

6622 THE GENETICS OF C-TYPE RNA TUMOR VIRUSES.

(E.) Wyke, J. A. (Dep. Tumor Virol., Imp. ancer Res. Fund Lab., London, England). *Int Rev ytol* 38:67-109, 1974.

n this extensive review the properties of C-type RNA umor viruses are discussed. Most findings are de- ived from *in vitro* experimentation. Since much more s known about avian RNA tumor viruses, these viruses sarcoma and leukemia) are discussed in more detail han the mammalian tumor viruses (sarcoma and leu- emia). The virus properties are discussed separate- y for each group of avian C-type RNA tumor viruses nd then compared. Interactions between host cells nd viruses, metabolic and physiologic functions, re- ults from inhibition studies, properties of comple- entation, and transformation, replication and morpho- gic findings are described. The concepts presented the section on mammalian viruses are more hypo- etical and more emphasis is put on morphology; some the parameters mentioned in the avian section are lso discussed. The section on the nature and ori- n of genomes of RNA tumor viruses is speculative. me hypotheses and several models for RNA tumor rus genome are discussed, evaluated critically in ght of experiments and weighed against each other. rther experiments should elucidate the relation- ip between viral and host genomes. (154 references)

6623 DNA TRANSFER AND VIRUS-CELL RELATIONSHIPS.

(E.) Melnick, J. L. (No affiliation), A. L. Boyd and J. S. Butel. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 31-54.

Three series of experiments were performed to evaluate the promise of the DNA transfer method for study of the transfer of genetic information between mammalian cells. In all three studies, total cellular DNA from one species was inoculated into tissue cultures of cells derived from another species. The first study involved the rescue of papovavirus SV40 by the passage of transformed cell DNA in permissive simian cells. The second study involved the ability of simian cell DNA to alter recipient hamster cells so that they could adsorb poliovirus. In the third study, heterologous SV40 pseudovirion production following inoculation of foreign DNA was investigated. In all three studies, total cellular DNA from one species was inoculated into tissue cultures of cells derived from another species; in all cases, something had happened to the foreign DNA once it was engulfed by the recipient cells. First, the SV40 genome was excised and replicated, resulting in the formation of progeny virus (efficiency of virus rescue, 50-80%). Second, the foreign (simian) DNA was at least transiently functional because a small fraction of the recipient hamster cells developed the capacity to attach poliovirions. The altered cells appeared to have uncoupled the phenomenon of attachment (and possibly penetration) from that of uncoating of poliovirus, since the adsorbed virus did not become eclipsed. Third, the inoculated foreign DNA was cleaved and encapsidated in SV40-infected cells, resulting in the formation of heterologous pseudovirions. Exhaustive quantitative studies are needed to determine the amount of foreign DNA that can be encapsidated and ultimately delivered to a new recipient cell by the pseudovirions and to contrast that with the amount of naked DNA taken up directly by a cell. To increase the extent of biological alterations in any type of transfer experiment, methodology must be developed to increase the amount of foreign DNA introduced into recipient cells. A more complex problem is the need for greater specificity in transfer studies: known nucleic acid sequences coding for delineated gene products must ultimately be employed. (81 references)

6624 RNA-DIRECTED DNA POLYMERASE—PROPERTIES AND FUNCTION IN ONCOGENIC RNA VIRUSES AND CELLS.

(E.) Green, M. (St. Louis Univ. Sch. Med., Mo.) and G. F. Gerhard. *Prog Nucleic Acid Res Mol Biol* 14:187-333, 1974.

Scientific work stimulated by the discovery, in 1970, of an RNA-directed DNA polymerase (RNA → DNA polymerase, reverse transcriptase) in oncornavirus particles is reported. Oncogenic RNA viruses and other viruses containing RNA → DNA polymerase are considered in relation to criteria for their classification, the viral 60-70S RNA genome and other nucleic acids, and biological properties. Endogenous RNA → DNA polymerase activity is described in terms of general properties and nature of the DNA product; in addition, oncornavirus DNA polymerase activity with natural DNA and synthetic homopolymer duplexes is noted. Purification, structural properties, and ribonuclease H activity of oncornavirus DNA polymerase are detailed, as are DNA synthesis with purified oncornavirus DNA polymerase and the mechanism of DNA synthesis from viral RNA. Comparison is made between the template specificity of oncornavirus DNA polymerase and that of bacterial and mammalian cell DNA polymerases. Other enzyme activities associated with oncornavirions are discussed. Data are presented on the inhibition of RNA → DNA polymerase and animal cell DNA polymerases by rifamycin derivatives, other ansamycins, synthetic polyribonucleotides, inhibitors that bind to the template, and other inhibitors. Evidence is reported for the *in vivo* function of viral RNA → DNA polymerase, including data prior to the discovery of RNA → DNA polymerase, infectious DNA in oncornavirus-transformed cells, polymerase-defective oncornaviruses, and detection and localization of virus-specific DNA in uninfected cells and in oncornavirus-infected and -transformed cells. Current views on the mechanism of virus replication and cell transformation by oncornaviruses are summarized. A discussion of the search for viral base sequences and RNA → DNA polymerase activity in cancer and normal cells includes the use of the DNA product of oncornavirus RNA → DNA polymerase to analyze human cancers for viral-related sequences, RNA → DNA synthesis in cancer and normal cells, and evidence of RNA → DNA polymerase activity in chicken embryo cells and amphibian oocytes. The role of oncornavirus genetic information and RNA → DNA polymerase in the etiology of neoplasia is analyzed. (412 references)

6625 BIOSYNTHESIS OF GLYCOLIPIDS IN VIRUS-TRANSFORMED CELLS.

(E.) Brady, R. O. (Natl. Inst. Neurol. Dis. Stroke, Bethesda, Md.) and P. H. Fishman. *Biochim Biophys Acta* 355(2): 121-148, 1974.

The biosynthesis of glycolipids in virus-transformed cells is reviewed with emphases on the ganglioside components and metabolism in established mouse cell lines, ganglioside patterns in virus-transformed mouse cell lines, metabolism of gangliosides in normal and virus-transformed mouse cells, ganglioside composition and metabolism in cells from other species, composition and metabolism of neutral glycolipids in transformed cells, the molecular basis of altered ganglioside metabolism in virus-transformed cells, and the relationship between altered ganglioside metabolism and tumorigenicity. Mouse cells contain a homologous series of gangliosides increasing in size from monosialosyldihexosylceramide to disialosyltetrahexosylceramide. After transformation by oncogenic DNA and RNA viruses as well as by chemical carcinogens and X-irradiation, there is a reduction in the oligosaccharide chains of the compounds due to specific blocks in ganglioside biosynthesis. The decreased glycosyltransferase activities observed in the tumorigenic virus-transformed mouse cells appear to be associated with the transformation process. Although the molecular basis of these changes is unknown and the relationship between these acidic membrane glycolipids and the behavior of cells in culture and *in vivo* is unclear, the discovery of an altered ganglioside metabolism in these cells may be an important implement to cancer research. (412 references)

6626 COMPARATIVE PROPERTIES OF PLANT TUMORS.

(E.) Hirth, L. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York, Academic Press, 1974) pp. 427-454.

Three types of plant tumors are examined in detail: those arising from the action of growth substances, those of genetic origin, and those induced by bacteria or viruses. Tumor calluses are obtained by regularly transplanting plant stems in medium containing auxin; in some cases cell proliferation gives rise to a voluminous callus that is able to grow in medium without auxin. Such "habituated" tissue induces a corresponding tumor when grafted onto a plant of the same genus. This type of tumor transformation is a relatively slow process compared with the fast virus-induced process (i.e., wound tumor virus) or of *Agrobacterium tumefaciens* (crown-gall)-induced tumors. Many tobacco plant species give rise to tumor-producing plants through cross-breeding. The transmission of wound virus and the characteristics of the induced tumor are detailed. Crown gall is discussed in relation to conditions of tumor induction, conditioning of plant cells, cytological properties of normal and transformed cells, and biochemistry of normal and tumor cells, including ability to synthesize new substances and nutritional needs. Differentiation, dedifferentiation, and cell multiplication are described for crown gall and habituated tobacco tissues. The nature of the tumor-inducing principle is examined for the case of crown gall for which the integration of exogenous genetic information has been confirmed. Whatever its origin, tumor transformation induces modifications related to regulation of synthesis of amino acids and growth regulators. (70 references)

6627 GENETICS AND BIOLOGY OF MURINE LEUKEMIA VIRUS.

(E.) McCarter, J. A. (No affiliation), P. K. Y. Wong and J. K. Ball. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974) pp. 259-277.

Although there is little published information on the genetics of murine leukemia viruses, studies that have been made in the murine system are reviewed and discussed in relation to similar studies of the avian RNA tumor viruses. The isolation and characterization of temperature-sensitive mutants of murine leukemia viruses are discussed in terms of models based on the concept that the genome consists of segments of single stranded RNA. Three possible models of the genome are postulated: 1) all segments are the same; 2) two segments are identical, the third different; and 3) all segments are different. There is as yet no definite answer from molecular biological studies as to the size of the genome of the RNA-containing tumor viruses. Despite the best efforts to isolate the RNA in intact form, there remains the possibility that large molecular weight RNA possesses a limited number of sites uniquely sensitive to the action of nucleases. Only two nicks would be sufficient to produce three fragments. To account for the observations that have been made, the nuclease-sensitive sites, if they exist, would have to be located so that equal sized pieces (30-40S) would be made; for unknown reasons, these would then have to associate with one another and with 4S-12S RNA to form the RNA structure of the mature virion. While this conclusion must be further evidenced, the accumulated evidence favors the segmentation of the RNA of the virion. (62 references)

6628 HERPESVIRUS ETIOLOGY OF ABNORMAL GROWTH.

(E.) Rapp, F. (No affiliation) and R. W. Koment. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974), pp. 183-205.

The possible relations of herpes viruses to human cancer are reviewed first by studying non-human herpes viruses in animal hosts and human herpes viruses studied in tissue culture or in non-human animal hosts. Frog adenocarcinoma, and lymphoma of chicken, guinea pig, rabbit and monkey are described in detail. Biological properties and isolation of three human herpes viruses, Epstein-Barr, herpes simplex and cytomegalovirus, are described including their transforming effects on cultured mammalian cells (mostly hamster embryo cells). The role of the herpes virus genome in oncogenesis through synthesis of antigens, nucleic acids, control of virus expression and induction of virus synthesis is presented. The involvement of latent herpes viruses in some forms of human cancer is predicted. (131 references)

6629 COMPARITIVE BIOLOGY OF MURINE AND AVIAN TUMOR VIRUSES.

(E.) Bentvelzen, P. (No affiliation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York: Academic Press, 1974) pp. 281-367.

The comparative biology of the avian and murine tumor viruses is reviewed, with emphasis on the interactions between host genome and the oncogenic RNA viruses. Although recent evidence indicated that the production of a DNA provirus is required for cellular transformation and the replication of an oncornavirus, the establishment of a somatic provirus is not the sole answer to the problem of the neoplastic conversion of cells by such viruses. Epigenetic factors appear to influence the transforming activity of the somatic provirus of the leukemia viruses strongly, the mouse mammary tumor system providing a good example of epigenetic control of the expression of somatic proviruses. It is unlikely that reverse transcriptase plays a role in the genetic transmission of oncornaviruses, although it may play a great role in the continuous release of infectious virus. It is postulated that strains of mice which have a low natural incidence of mammary cancer have a repressor which inhibits the transcription of their own germinal provirus and that of other strains in various hybrids; there is strong evidence that there are both site-specific and overall repressors. In chickens, a dominant mendelian factor exists which causes the release of the group-specific antigen of the avian tumor viruses; this factor probably reflects a mutation in a site-specific regulatory gene of the germinal provirus. A recessive gene causing release of the viral antigen and susceptibility to superinfection may be due to a mutation in the overall regulatory gene. It is likely that different oncogenes with their associated regulatory elements are responsible for strain differences in tumor-susceptibility in mice. Although there is little evidence for the infectious nature of cancer in man, the human cancer problem will likely benefit from investigation into the relationships between virus and host genome in animal models and study of the immunobiology of naturally occurring oncornaviruses. (650 references)

6630 SIMIAN HERPESVIRUSES AND NEOPLASIA.

(E.) Deinhardt, F. W. (Univ. Illinois Med. Cent., Chicago), L. A. Falk and L. G. Wolfe. *Adv Cancer Res* 19:167-205, 1974.

The role of simian herpesviruses in neoplasia is reviewed because some are oncogenic in nonhuman primates and are similar to human viruses believed to be oncogenic, (i.e. Epstein-Barr virus). The isolation, characterization *in vivo* and *in vitro*, immunology, epidemiology, natural infection, experimental transmission and symptomatology of herpes viruses is presented with emphasis on *herpesvirus saimiri* (HVS) and *herpesvirus ateles* (HVA). Oncogenicity of these herpes viruses to non-human primates is described and related to the study and possible preventive therapy of similar disease in man. It is concluded that the importance of non-human primate model systems lies in the opportunities they offer for studying the pathogenetic mechanisms of lymphoproliferative diseases induced by herpesviruses. (121 references)

6631 A NEW LOOK AT THE MODE OF ACTION OF POLYOMA AND RELATED TUMOR VIRUSES.

(E.) Weil, R. (No affiliation), C. Salomon, E. May and P. May. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 455-498.

It is proposed that polyoma (Py) and related DNA viruses (SV40 and papilloma viruses) act primarily as endogenous mitogens and that malignant transformation is not directly determined by viral genes but is the outcome of a combination of prolonged virus-induced cell proliferation and cytogenetic instability. This theory is contrasted with the hypothesis that viral-induced cell transformation involves a process akin to lysogenic conversion of bacteria. Earlier and recent experimental evidence leading to the theory of mitogenic stimulation is summarized. The mechanism involved in the virus-specific mitogenic action is discussed in relation to cell transformation in tissue culture and tumor formation *in vivo*. It is noted that the presence of integrated Py or SV40 viral DNA is apparently not required to maintain malignancy once it has been established. Similarities between malignant transformation induced by oncogenic DNA viruses and chemical carcinogens are briefly discussed. The long-range potential of Py and related viruses for biological research and medicine resides in their usefulness for studying mitosis, transcription, and processing of mammalian RNA and possibly as model systems for gaining insight into the mechanisms involved in embryonic determination and differentiation. (147 references)

6632 CLINICAL VALUE OF TUMOUR-ASSOCIATED ANTIGENS. *(E.)* Neville, A. M. (Inst. Cancer Res., Royal Cancer Hosp., London, England). *J Clin Pathol* 27(Suppl. 7):119-126, 1974.

The detection of tumor-associated antigens in body fluids could lead to the facilitation of tumor diagnosis and to advances in the field of tumor histopathology. Macrophage electrophoretic mobility test systems can be used to detect the presence of the basic cancer protein of human malignancies. The usefulness of this test is extensive (although some exceptions are specified), even detecting lymphocytic sensitivity prior to the tumor's becoming clinically detectable. Alpha-fetoprotein (AFP) is detected most commonly by gel diffusion methods, raised AFP levels being remarkably specific for hepatocellular carcinoma and malignant teratomas; positive assays occur also when other malignancies metastasize to the liver. Radioimmunoassay shows raised AFP levels in hepatocellular carcinoma patients, but also shows raised values in cirrhosis and viral hepatitis patients. The assay of plasma carcinoembryonic antigen (CEA) is clinically valuable in the diagnosis of carcinomas of the gastrointestinal tract, pancreas and bronchus and of some value in the assessment of neuroblastoma and possibly mammary carcinoma. Inasmuch as early tumors are associated with low plasma CEA levels, estimation is of little or no value as a screening procedure. Urinary CEA levels are elevated in patients with urothelial carcinomas and when the urinary tract is infiltrated by hypernephroma and prostatic carcinoma; well-differentiated tumors *in situ* give rise to high urinary CEA levels even when urinary exfoliative cytology is negative. Plasma CEA determination is used to detect residual neoplastic disease (postoperatively) and recurrent or metastatic development prior to clinical diagnosis. Plasma CEA levels, high with extravesical or pelvic metastases, combined with urinary determinations may be useful to detect recurrences of urothelial carcinomas. There is also evidence from human and experimental work that the development of hepatomas may be preceded by rises in AFP serum levels and that recurrences are associated with coincident rises in AFP values. Both AFP and CEA have been employed as biochemical index substances to assess the effects of chemotherapy; patients with raised AFP levels have more aggressive teratocarcinomas while the majority of AFP-negative malignant teratomas respond to therapy. Measurement of as many tumor-related principles as possible could result in profiles useful for early detection, differential diagnosis, discovery of residual or recurrent growth, and monitoring therapy. (85 references)

6633 PROSPECTS FOR VACCINES AGAINST CANCER. *(E.)* Hilleman, M. R. (No affilation). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch. (New York: Academic Press, 1974) pp. 549-560.

The present status of immunologic control of cancer is reviewed. A concerted effort is being directed to increasing host resistance to cancer either by preventing or limiting infection with oncogenic vaccines, or by increasing resistance to cancer on tumor-antigen or nonspecific immunostimulatory bases. Viral studies in animals have yielded positive results in immunizing against the avian-leukemia-sarcoma complex, against the murine leukemia-sarcoma complex, against adenoviruses that are oncogenic for animals, and against SV40, polyoma, and myxomatosis viruses. The feline leukemia-sarcoma model may be useful in evaluating live, killed, and subunit vaccines for prophylactic and therapeutic efficacy and thus provide information that can be applied to the development of vaccines for man. Results with Marek's disease vaccine show that a vaccine needs only to limit the ability of virulent virus to express itself clinically as cancer; the vaccine need not prevent infection with the virulent virus. Of the five known oncogenic viruses of nonhuman primates, herpesvirus saimiri and herpesvirus ateles are excellent models for studying immunologic interruption of cancer in primate species using live, killed, or subunit viral vaccines. The only candidate human oncogenic viruses worthy of vaccine consideration at this time are those of herpes simplex. The technology for preparing highly purified subunit antigens and the availability of immunologic adjuvants should facilitate research on this group of infections. Practical application of tumor-specific immunization might be limited to use of the patients's own tumor tissue. However, a common factor in tumor antigens induced by a single virus, and to some degree in spontaneous tumor of similar type, might permit a wider application of this technique. The use of nonspecific measures for tumor immunity appears best directed to stimulating the accumulation of mononuclear cells (lymphocytes and macrophages) that are active in delayed hypersensitivity. (9 references)

6634 IMMUNOLOGICAL PATTERNS OF VIRUS-TRANSFORMED NEOPLASTIC CELLS. *(E.)* Klein, G. (Inst. Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden). In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974), pp. 501-512.

Two categories of antigens in virus-transformed cells are discussed; the intranuclear virus induced antigens and the surface membrane antigens. The former type includes polyoma-, simian virus 40- and adenovirus-associated antigens plus a more recently discovered soluble antigen associated with the Epstein-Barr virus; this latter antigen is extensively reviewed leading to the conclusion that nuclear antigens provide clear evidence of viral genome "footprints". The discovery of such a "footprint" in human acute myeloblastic leukemic cells is described. Tumor-associated transplantation antigens (TATA) are reviewed in relation to such questions as the universal appearance of TATA in all virus-induced tumors, direct involvement of TATA in neoplastic change and the effects of TATA on host immune surveillance. The variable immune and carcinogenic response to virus-induced TATA in various animal species is described. It is concluded that specific, genetically-determined responsiveness has evolved to deal with common events of potential neoplastic transformation, occurring upon exposure of the natural host to potentially oncogenic viruses. (47 references)

6635 IMMUNOLOGIC MODIFICATION: A BASIC SURVIVAL MECHANISM. *(E.)* Jacobs, B. B. (Am. Med. Cent. Denver, Spivak, Col.), and D. E. Uphoff. *Science* 185(4151):582-587, 1974.

Alterations in the phenotypic expression of immunogenicity often contribute to the survival of cells or tissues that otherwise would be eliminated by an immune reaction. Cellular antigens and the responding organism interact in a dynamic way, both participating in the end result, be it survival or death of grafted cells. Phenotypic alteration of cell surface antigens may be due to modification of phenotype rather than genetic variants, as seen in example (a-d). (a) Original phenotypic traits, e.g. antigen expression, not shown by tissue culture cell lines may be recovered spontaneously or by changing the culture conditions. (b) Modification of immungenicity may be associated with the physiological environment. (c) The expression of cellular antigens and serum proteins is influenced by the stage of differentiation of the organism and the presence or absence of neoplasia, as has been noted with the appearance of carcinoembryonic antigen, alpha fetoprotein and gamma fetoprotein. (d) Antibody-induced changes of antigen expression occur *in vivo* and *in vitro*, as illustrated by the disappearance of mouse thymus leukemia antigens on leukemic cells and normal thymocytes during exposure to the thymus leukemia antibody and subsequent reappearance when antibodies are not present. Allograft survival can be extended by *in vitro* incubation of donor tissue with antigenic material or short-term organ culture explantation. Prior treatment of donors *in vivo* or donor tissue *in vitro* with various antisera has extended allograft survival, and the immunogenicity of tumors was decreased by a single overnight passage in allogeneic mice of the recipient strain. Host modifications subsequent to allograft exposure may operate to allow for survival of a subsequent graft. Interference with the host's immune response by enhancing antibody and alterations in the graft itself contribute to the host's adaptation. Graft modification may be induced by passage through an unresponsive host, and reversible graft adaptations can occur during growth in nonspecifically and specifically immunoincompetent recipients. Allografts may also be conditioned by residence in immunologically privileged sites, the successful maternal-fetal relationship being attributable to the placenta being a privileged site. Immunotherapy of tumors could utilize methods which would both increase the immunogenicity of the target cells and increase the ability of the host to recognize these cells; such an approach has been successfully applied using combined treatment with BCG and neuraminidase-treated tumor cells. (73 references)

6636 AUTOIMMUNITY AND NEOPLASIA: THE POSSIBLE ROLE OF C-TYPE VIRUSES. *(E.)* Levy, J. A. (Cancer Res. Inst., Univ. California, San Francisco). *Am J Clin Pathol* 62(2):258-280, 1974.

Viruses have been implicated in several autoimmune diseases in animals (e.g., Aleutian disease in mink, infectious anemia in horses, and lymphochoriomenigitis in mice) and in man (e.g., Goodpasture's syndrome, Coombs-positive hemolytic anemia). C-type RNA tumor viruses induce neoplasia in a variety of animal species, but their role in human malignancy is unknown. An unusual C-type virus has been isolated from New Zealand black (NZB) mice, which have an inherited disease syndrome involving both autoimmune phenomena and neoplasia. This virus cannot be propagated in mouse cells but does not grow in cells foreign to the host species. For this reason, it has been termed a xenotropic murine leukemia virus. Similar viruses have been detected with less frequency and in lower titers in NIH Swiss and other mouse strains. Since horizontal infection in the host is unlikely, this xenotropic virus must represent an endogenous virus of mice. Because of its prevalence in NZB mice, particularly during embryonic life, it is suggested that it plays a role in the pathogenesis of both autoimmune disease and malignancy. It is likely that the host genome of each animal species could contain genes for two classes of endogenous C-type viruses: xenotropic and ecotropic (i.e., those that can propagate in the cells of the host species, like MLV). Autoimmune diseases of man resemble NZB disease with regard to genetic susceptibility, immune deficiency, and thymic injury. It is possible that the C-type viruses observed in certain human tumors may, in fact, be endogenous xenotropic viruses which await isolation and cultivation in the appropriate cells from nonhuman species. (185 references)

6637 IMMUNOLOGICAL PARALYSIS ASSOCIATED WITH TUMOR GROWTH. *(Rus.)* Shats, V. Ia. (N. N. Petrov Sci. Res. Inst. Oncol., Moscow, USSR). *Vopr Onkol* 20(11):85-95, 1974.

Prior literature is reviewed on immunological paralysis (IP) as a factor affecting tumor growth. IP as a form of tolerance is the result of a general nonspecific immunodepression induced by carcinogens together with an effect of high levels of tumor antigens, a suppressed antigenic difference between tumor cells and normal tissues acting as a contributing factor. In adult animals, tumor tolerance can be induced by the action of injected antigens. Injecting large volumes of blood from DBA mice into BALB/cAn mice can inhibit the growth of subsequent transplants of DBA tumor; however, similar experiments with transplants of other tumor lines result in the growth of the allogenic tumor in the recipient. Immunization can be obtained by injecting a suspension of allogenic lymphoid cells, tumor antigens or embryonic tissue. Tolerance to tumor antigens is sometimes observed with tumors induced by viruses. The methods of recognizing tolerance to tumor growth include: the slow supersensitization reaction generally caused by injecting s.c. tumor-antigen-containing substrates into tumor-bearing animals and humans; blast transformation of lymphocytes; interaction of lymphocytes and tumor cells *in vitro*; immunocyte effect on the test for adoptive immunity; the reaction of humoral antibodies. It is suggested that IP associated with tumor growth should not be overestimated. There are no completely reliable methods of recognizing it. Moreover, the outpouring of excessive amounts of antigens, an important condition of inducing tolerance, usually occurs at later stages of tumor growth when the tumor reaches a substantial size. It is assumed that IP is a possible but not essential part of the immunological response to tumor growth. (121 references)

6638 T AND B LYMPHOCYTIC NATURE OF LEUKEMIAS AND LYMPHOSARCOMAS: A NEW BUT STILL UNCERTAIN PARAMETER FOR THEIR CLASSIFICATION. *(E.)*

Mathé, G. (Inst. Cancerologie Immunogenetique, I.N.S.E.R.M., Villejuif, France) and D. Belpomme. *Biomedicine* 20(2):81-85, 1974.

A variety of methods and techniques have been used to distinguish T lymphocytes, B lymphocytes, and macrophages; a critique of immunologic procedures is presented. Immunological investigation of human leukemia and hematosarcoma (LH) cells has considerably modified the conventional classification of several diseases. For some diseases the reclassification is either different from morphologic grouping or as yet leaves some entities unclassified. Chronic lymphoid leukemia appears to be composed of differentiated lymphocytes of the B series, as does primary macroglobulinemia. Generally considered as a histiocytic malignant proliferation, Sézary's disease is composed of T lymphocytes, as are mycosis fungoides, infectious mononucleosis and Burkitt's lymphoma. Of the common diffuse lymphosarcomas, some are of a T cell nature and some B cell; early leukemia lymphosarcoma (usually in children) is T cell while nodular lymphosarcomas are probably B cell until the individual disorder becomes diffuse and is either B or T cell in nature. Some reticulosarcomas may be of a lymphocytic nature, but their immunological characterization is necessary for such a diagnosis to be accepted. While a fundamental T lymphocyte abnormality has been suspected as a cause of the defect in cell mediated immunity in Hodgkin's disease, the Reed-Sternberg cells which proliferate in this disease may be of B lymphocytic and histiocytic nature. Preliminary evidence indicates that the leukemia cells in acute lymphoid leukemia (ALL) may be T cells, but this awaits confirmation. In 20 ALL cases tested, T markers were present in 5 cases and there were no markers in 15 cases. Chronic lymphoid leukemia cells that carry receptors are B types. Uncertain results require a cautious approach to immunologic classification of human LH, but such a classification should be considered relative to clinical and histologic aspects, including prognosis and therapy.(51 references

6639 TUMOR IMMUNITY: THE LYMPHOID SYSTEM AND EXPERIMENTAL NEOPLASIAS. *(E.)*

Davies, A. J. S. (Chester Beatty Res. Inst., London, England), B. J. Weston, R. L. Carter and T. A. Connors. *J Clin Pathol* 27(Suppl. 7):72-76, 1974.

Early studies of the role of the thymus in tumor immunity suggest two hypotheses for mechanisms of action: secretion of a hormone by the organ, and production by the organ of a population of immunologically reactive cells. It is too early to evaluate fully the new evidence for the humoral factor, more effort having gone into establishing the cellular hypothesis. Depending on the species studied, studies of thymus function support or repudiate the hypothesis that cellular and humoral immunity are separate entities operated by separate cell populations. Extrapolation between species is hazardous because of such variations in immune mechanisms. A complex array of cells is probably involved in some kinds of immune response. Various types of experiments with T cell-deprived mice show that although the growth of transplanted tumors can be affected by the immune response, this is by no means a widespread phenomenon. The effectiveness of immune control can be affected by the tumor mass, and T cells can somehow control tumor growth. In situations in which chemotherapeutic treatment of a tumor is attempted in human beings, the immune response which was previously ineffective in controlling tumor growth may be successful in eradicating or controlling the reduced tumor mass. A vigorous immune response may be ineffective because its humoral and cellular components are self-effacing. Immunological exhaustion may arise after which no further augmentation of an antitumor response takes place. Thus, while tumor growth can be progressive and unrestricted, the growth of an antitumor response probably cannot. (33 references)

6640 LYMPHOCYTE-MEDIATED IMMUNE RESPONSES IN NEOPLASIA. *(E.)*

Pilch, Y. H. (Dep. Surg., Univ. Calif., Los Angeles) and S. H. Golub. *Am J Clin Pathol* 62(2):184-211, 1974.

Different approaches for the study of cell-mediated immunity to tumors are evaluated with respect to yield of useful information and inherent pitfalls. Among the methods available for the assessment of cell-mediated immunity are assays of lymphocyte-mediated cytotoxicity, blastogenesis, the detection of lymphokines and delayed cutaneous hypersensitivity reactions, all of which offer advantages for the study of cell-mediated immunity. As to the nature of the cells mediating the tumor-specific immune responses, there is evidence implicating both T and B lymphocytes as well as macrophages; no major cell component of the immune system can therefore be excluded from involvement in tumor-specific immunity. Two types of interactions which would result in inhibition of the cytotoxic effects of lymphocytes on tumor cells can be envisioned: "blocking" activity, and "desensitization". Similarly, two types of serum effects which promote cytotoxicity have been described: "unblocking", and "antibody-dependent cellular cytotoxicity". Numerous studies have shown that tumor-specific transplantation immunity can be adoptively transferred to previously untreated hosts by the administration of syngeneic lymphoid cells obtained from animals previously immunized with the tumor in question. Additional studies have implicated two lymphoid extracts in the mediation of cellular immune responses to tumors: "immune" RNA and "transfer factor". The former offers exciting possibilities as a new immunotherapeutic modality for the treatment of cancer but more evidence is needed before the eventual role of a transfer factor in the immunotherapy of cancer can be determined. (154 references)

6641 THE ULTRASTRUCTURE OF THE LOCAL CELLULAR REACTION TO NEOPLASIA. *(E.)*

Car, I. (Dep. Pathol., Univ. Sheffield, England) and J. C. Underwood. *Int Rev Cytol* 37:329-347, 1974.

An intrinsic part of the neoplastic process is the abnormal relationship between neoplastic cells and the surrounding non-neoplastic cells. A neoplasm stimulates a lymphoreticular reaction, a vascular reaction, a fibrous reaction, or a banal inflammatory response. Study of the lymphoreticular response is hampered by experimental difficulties. Much of the ultrastructural work that has been done in the study of the lymphoreticular response refers to the *in vitro* situation or the peritoneal cavity and therefore must be applied with caution to solid tumors since it might not apply in the *in vivo* situation. The lymphoreticular reaction involves both macrophage and lymphocyte activities. There is evidence that macrophages ingest and destroy intact human cells and they may be aided by lymphocytes which either coat the macrophages with cytophilic antibodies or deposit antibodies on the tumor cells, either as cytotoxin or as a mere opsonin to faciliate phagocytosis. It is uncertain whether lymphoreticular cells in neoplasms are locally or marrow derived, whether they recirculate, and whether there is any form of intercellular cooperation between lymphocytes and macrophages. In regressing animal neoplasms macrophages phagocytose neoplastic cells but the evidence for this defensive activity of lymphoreticular cells in established human tumors, other than Hodgkins disease, is scanty. Neoplastic cells also stimulate proliferation of vascular endothelial cells and formation of capillary sprouts which quite rapidly form sinusoidal vascular networks in which fenestrated endothelium is often present. There is little doubt that this reaction is mediated by the release of a soluble factor by the neoplastic cells. Tests showed that this factor responsible for angiogenesis contained about 25% RNA, 10% protein, and 50% carbohydrate and the rest presumably lipid. It seemed that the effect of this factor is at least semispecific and not merely due to the induction of an inflammatory reaction. The growth of neoplastic cells is also associated with proliferation of adjacent fibroblasts but this has been studied in less detail than the vascular and lymphoreticular reaction and is presumably similar and possibly mediated in the same way as the vascular reaction. (56 references)

6642 CHROMOSOME ALTERATION IN THE COURSE OF SERIAL TRANSPLANTATIONS OF EXPERIMENTAL TUMORS AND AGING OF TUMOR STEMLINE CELLS. *(E.)*

Yosida, T. H. (Natl. Inst. Genet., Japan). *Recent Results Cancer Res* 44:86-93, 1974.

According to the concept of tumor stemline in transplantable tumors, a tumor has a cell lineage dividing regularly through cell generations with a characteristic chromosome constitution. The karyotype of the stemline, however, is not always constant but may change during serial transplantations. Data are presented on karyotype alterations in the Yoshida rat sarcoma and the MY mouse sarcoma, in mouse plasma cell tumors, and in Shay's rat chloroma. The karyotype alteration is an effect of aging. A possible explanation for the limited life span of the tumor stemline, and cell degeneration after a certain period of cell multiplication, is an unfavorable accumulation of genes in stemline cells during the progress of transplant generations. A chromosome alteration may be necessary to break this tendency of aged cells to degenerate. A functional change in cells following a ploidy shift or chromosome structural change in the tumor stemline has been demonstrated in many tumor strains. A change in gene function following the chromosomal alteration may play an important role in the rejuvenation of the aged stemline cells. (32 references)

6643 CHROMOSOME CHANGES IN HUMAN MALIGNANT TUMORS: AN EVALUATION. *(E.)*

Sandberg, A. A. (No affiliation). *Recent Results Cancer Res* 44:75-85, 1974.

Published chromosomal findings are evaluated for relevance to specificity of karyotypic alterations in human neoplasia, the role of chromosomal changes (including markers) in the causation of human cancer and leukemia, and possible future application of cytogenetic data in the diagnosis and treatment of these diseases. On the evidence available in the literature, the chromosomal changes in most instances of neoplasia are epiphenomena. While chromosomal aberrations may play a part in tumor progression once the neoplastic process is established, they are not involved in the initial causation of human cancer and leukemia. The presence of double-minute chromosomes in neurogenic tumors, and occasionally in other types of cancers, may be indicative of the presence of a virus in such tumors. The genesis of certain characteristic markers in some tumors may yield a clue as to the possible etiology of the tumor (e.g., C-type chromosome in Burkitt's lymphoma). Furthermore, the biologic behavior of a tumor may be related to the presence of such marker chromosomes or to a unique aneuploidy, which may also play a role in the metastatic capability of the cancer. The presence of marker chromosomes of distinct morphology may be related to the cause of the neoplasia and thus provide clues as to the possible etiology of the various human cancers. (63 references)

6644 ORDERED AND SPECIFIC PATTERN OF GENE EXPRESSION IN DIFFERENTIATING AND IN NEOPLASTIC CELLS. *(E.)* Weber, G. (Indiana Univ. Sch. Med., Indianapolis). *Proc Fourth Int Symp Princess Takamatsu Cancer Res Fund, Tokyo* 151-180, 1973.

Recent advances made by application of the molecular correlation concept as an experimental and conceptual method in different metabolic areas are reviewed and analyzed and the separation of the biochemical pattern of hepatic neoplasia from that of differentiating liver is demonstrated. The linking of newly discovered aspects of the biochemical imbalance in neoplastic cells with malignant transformation and with the degrees of expression of malignancy is evaluated. The biochemical and enzymatic alterations that occur in all tumors are characteristic of the reprogramming of gene expression that occurs in the malignant transformation. The reprogramming of gene expression in malignancy is also expressed in the shift of isozyme pattern where there emerges a predominance of the low K_m isozymes. Concurrently, the high K_m regulatory enzymes diminish. These alterations are the molecular basis, in part, for the decreased responsiveness of the cancer cells to regulatory signals. The ordered pattern of gene expression as manifested in the metabolic and enzymatic imbalance discovered in the hepatoma spectrum is specific to neoplasia. There is no similar pattern in fetal, differentiating, regenerating, or normal adult liver. The neoplastic pattern of gene expression is both quantitatively and qualitatively different from that of the various control tissues. The biochemical imbalance confers selective biological advantages by the progressive predominance of the synthetic pathway over the declining catabolic one in pyrimidine, purine, and nucleic acid metabolism. An evaluation of extensive evidence has led to the conclusion that what is important about cancer is ordered and that the diversity and the random element in cancer are not important. (31 references)

6645 NEOPLASIA AND DIFFERENTIATION AS TRANSLATIONAL FUNCTIONS. *(E.)* Pitot, H. C. (Univ. Wisconsin Sch. Med., Madison). In: *Developmental Aspects of Carcinogenesis and Immunity* edited by T. J. King (New York: Academic Press, Inc., 1974) pp. 79-88.

Messenger RNA stability in neoplasia and during differentiation is reviewed in relation to the concepts of developmental biology. It has been argued that messenger RNA template lifetimes and turnover may be the major mechanism whereby cellular differentiation is effected. Other studies have indicated that, from a theoretical standpoint, the malignant transformation may well result from altered messenger RNA stability; the most malignant tumors having very stable templates of messenger RNAs whose products are directly involved in cellular replication. It is suggested that neoplasia may be related to an abnormality in differentiation through abnormal template stabilization and translational control. (42 references)

6646 THE HISTOLOGY OF RARE HUMAN TUMORS AND THEIR RELATIONSHIPS TO GENERAL TUMOR PATHOLOGY. *(E.)* Laumonier, R. (No affiliation) and G. Chomette. *Recent Results Cancer Res* 44:69-74, 1974.

Human tumors may be rare with respect to statistical rate, morphology, or evolution. The pathological principles which relate to these different concepts of rarity include etiological factors, proliferation of tumor clones, and host reactions. Etiological factors are involved in statistical rate as exemplified by hereditary trait in retinoblastoma, congenital colon polyposis, Peutz Jehgers syndrome, and certain phakomatoses; by asbestos-induced pleural mesothelioma; by irradiation-induced sarcoma in carcinoma tissue; by parasite-induced papillomas and carcinoma; and by possible virus-induced African hematosarcoma. Morphological rarity, and to a certain extent developmental rarity, are the consequence of the proliferation of an unusual cell clone. Such clones may originate from embryological structures as exemplified by neuroblastomas, renal blastomas, genital blastomas; or from adult tissues as exemplified by carcinoma of the small intestine, medullary carcinoma of the thyroid, α-fetoprotein-producing hepatoma, non-secreting carcinoid tumor, hypoglycemia-producing mesenchymoma, and stomach myoid tumor. Involvement of host reaction in rare tumors may be related to secretions of neoplastic clones as in stromal amyloidosis, medullary thyroid carcinoma, calcospherites in papillary carcinoma, and hyperelastogenesis of mesenteric arterioles in carcinoid tumor; or to fibroblastic modifications of tumors as in the simultaneous proliferation of epithelial and mesenchymatous clones in thyroid carcinosarcoma, and in the transformation of the fibroepithelioma of Pinkus to a basal cell carcinoma. Host reaction may also be related to defense against clonal proliferation as in medullary carcinoma of the breast or tuberculoid histiocyte hyperplasia of satellite lymph nodes. Choriocarcinoma is presented as an example of a tumor which is rare statistically, morphologically, and developmentally. It is suggested that improvements in histology, such as in immunohistology, may bring about a better understanding of tumor rarities and of tumors in general. (14 references)

6647 ELECTRON MICROSCOPY OF ONCOCYTOMAS AND CARCINOID TUMORS. *(E.)* Schulz, H. (No affiliation). *Recent Results Cancer Res* 44:63-68, 1974.

Electron microscopic examination of oncocytes and oncocytomas have shown that oncocytes differ from other epithelial cells in their excessive proliferation of abnormal mitochondria. Oncocytic transformation of epithelial cells is regarded not as a degenerative process, but rather as a re-differentiation which leads to increased but unbalanced metabolic activity. For example, oncocytic adenomas and Hürthle-cell carcinomas of the human thyroid gland contain an abundance of oxidative enzyme systems in the Askanazy cells, and such cells can be viewed as functioning efficiently. Nevertheless, these cells are distinctly different in their function from normal thyroid cells. Since the enzyme systems required for the storage of iodine are missing in these cells and they are therefore unable to produce thyroid hormone, it may be assumed that overstimulation or abnormal stimulation of the oncocytic epithelial cells occurs. Unlike normal mitochondria, oncocyte mitochondria produce only very small quantities of ATP. Apparently, the oncocytes attempt to compensate for this functional defect by providing a greater number of mitochondria. Thus, oncocytic transformation must be regarded as the consequence of an acquired disturbance of the mitochondrial enzyme organization. It is possible to produce oncocyte tubuli and typical oncocytomas experimentally in the kidneys of rats treated with N-nitrosomorpholine (NNM). Intramitochondrial accumulation of glycogen, as found in some human oncocytomas, was not, however, seen in the NMM-induced oncocytomas. Carcinoids, which favor the intestinal tract and may metastasize in the regional lymph nodes and in other organs of the abdominal cavity, develop from enterochromaffine cells and produce a vasoactive substance, 5-hydroxytryptamine (5-HT). The 5-HT is associated with the granules of the carcinoid cells. Bronchial carcinoid is a solid adenoma, characterized by high degree of cell differentiation and the presence of granules shown cytochemically to contain unsubstituted catecholamines and indolamines. Under the electron microscope, the specific granules of carcinoid tumors look very similar to the specific cytoplasmic granules of various endocrine organs, including pheochrome granules of the suprarenal medulla, granules of A-cells and B-cells in islets of the pancreas, the neuro-secretory elementary granules, the specific granulations of the cells of the anterior lobe of the hypophysis, and the granules of the calcitonin-producing C-cells of the thyroid gland. (36 references)

6648 POLYPS, POLYPOSIS AND CANCER. *(Fr.)* Guivarc'h, M. (No affiliation). *Rev Prat* 23(25):2333-2339, 1973.

Malignancy develops in 20-30% of solitary polyps and in about 80% of multiple polyps of the colon and rectum. Since malignant transformation can begin at a single point on the tumor, only histological examination of the entire tumor can establish whether the tumor is benign or malignant. A transformed tumor has nuclear and architectural anomalies, increased rate of mitosis, and cells with a basophilic cytoplasm. Gross signs of malignancy include multiple lesions, diameters exceeding 1 cm, rapid growth, hardness of the surface, and irregularity of the base of implantation. Such signs, together with radiographic, endoscopic, and arteriographic evidence can be used to judge the optimum extent of excision prior to histologic examination. After limited excision recurrences occur in 22% to 41% and require careful follow-up. Isolated polyps in juveniles are of a very different histologic type from adenomatous polyps and never develop into cancer. Villous tumors degenerate into cancer in about half the cases. Malignant transformation can occur in all forms, but especially in large sessile lesions. Gross indications of malignancy are hard villous tumors or those ulcerated on the surface which bleed on contact. After incomplete excision about 20-25% recur. Adenomatous rectocolonic polyps account for 80% of multiple polyps; these invariably undergo transformation by the age of 40. The polyps generally begin to appear around puberty but are often not diagnosed until later. Tumors of Gardner's syndrome are a subgroup of multiple adenomatous rectocolonic polyps, and have the same potential of malignancy. Tumors of Peutz-Jeghers' syndrome constitute about 10% of multiple polyps and very rarely become malignant. Disseminated polyposis in juveniles accounts for about 10% of multiple polyps and never undergoes transformation. Polyps of Cronkhite Canada syndrome also never become cancerous. In cases of adenomatous polyps, preventive total colectomy is the only sure means of avoiding malignant transformation, and it must be followed up by careful surveillance of the remaining rectum. (No references)

6649 THE ANATOMICAL PRECURSOR OF COLORECTAL CARCINOMA. *(E.)* Fenoglio, C. M. (Coll. Physicians Surg., Columbia Univ., New York, N.Y.) and N. Lane. *Cancer* 34(3/Suppl.):819-823, 1974.

A commentary is presented on the moderately or well-differentiated adenocarcinomas which comprise the vast majority of colorectal carcinomas. The very common hyperplastic polyp is not a neoplasm and is unrelated to either adenoma or carcinoma. Adenomas, which are only 1/10 as common, may occur grossly as adenomatous polyps or papillary adenomas. They are true neoplasms, non-invasive and thought of as benign. Depending on size, and probably related to a sessile mode of growth, intramucosal carcinoma and/or "early" invasive cancer may be observed in adenomas. On the other hand, although microscopic examination has been performed on many thousands of minute mucosal lesions (5 mm or less), "early" cancer, defined as intramucosal carcinoma with or without microinvasion, does not seem to occur unassociated with adenoma. Because colorectal carcinoma is so common, it should be easy to find many examples of "early" cancer as defined above if the majority of ordinary adenocarcinomas arose directly from normal crypts of Lieberkuhn. The apparent non-existence of small foci of intramucosal carcinoma, with or without microinvasion, in normal mucosa, and their frequency in adenomas, are two fundamental pathologic facts. They seem to disprove the proposition that cancer cells ordinarily arise *de novo* from the normal cells of the crypt of Lieberkuhn without the interposition of a stage in the neoplastic process that is recognized as adenoma. While adenomas may be observed in any size or shape, it is especially the larger ones that are statistically precancerous lesions. It is concluded that the systematic detection of adenomas is meaningful in terms of preventive medicine, and that their removal is the most assured way of decreasing the incidence of colorectal carcinoma. (15 references)

6650 PERSPECTIVES ON THE EPIDEMIOLOGY OF CERVICAL CANCER WITH SPECIAL REFERENCE TO THE HERPESVIRUS HYPOTHESIS.

(E.) Kessler, I. I. (Sch. Hyg. Public Health, John Hopkins Univ., Baltimore, Md.). *Cancer Res* 34(5):1091-1110, 1974.

Recent data from Baltimore and elsewhere show that the age-adjusted incidence of cervical cancer in the U.S. has approximately doubled since 1950. This increase is attributable entirely to *in situ* cancer in women below age 50. Among older women the disease (primarily invasive) has declined in frequency; among younger women a decrease in invasive cancer has been paralleled by an even greater increase in *in situ* cancer incidence. These time trends are relevant to the herpesvirus hypothesis of human cervical carcinoma. An important unresolved problem is the biological behavior of cervical dysplasia and carcinoma *in situ*. If *in situ* lesions invariably progress to invasion then, under the herpesvirus hypothesis, one would expect evidence of prior herpetic infection in similar proportions of women with these two conditions; if only a fraction of *in situ* carcinomas invade, not all women with these lesions would be expected to demonstrate the presence of viral material. An analogous situation obtains with regard to evaluating serological findings in women with cervical dysplasia and cancer. That only about one cervical cancer in four is detected in an invasive stage today complicates methodologically sound testing of etiological hypotheses. Evidence on coital and penile risk factors is reviewed together with evidence favoring the herpesvirus theory. Despite differences in techniques and in types of patients selected, a positive association between cervical cancer and herpes simplex virus-2 (HSV-2) antibodies is clearly evident from most seroepidemiologic studies. A collaborative prospective study should make possible definite conclusions on the temporal sequence of the observed association between HSV-2 and the histopathologic stages of cervical neoplasia, from dysplasia to *in situ* cancer to invasive cancer. (103 references)

6651 EPIDEMIOLOGIC ASPECTS OF MAMMARY TUMOURS.

(E.) Cole, P. (Harvard Sch. Public Health, Boston, Mass.). *Proc Fifth Int Symp Biol Characterization Hum Tumors* 1:157-163, 1974.

The epidemiology of mammary tumors is reviewed with special attention given to those descriptive features related to child-bearing. Two strongly entrenched ideas, that lactation and high parity protect against breast cancer, have been shown to be wrong or the association noncausal. A new and stronger risk indicator, age at first birth, has emerged, although the association has not been shown to be causal. Age incidence curves suggest breast cancer has a dual etiology or, at least, that there are two periods in a woman's life, possibly adoloscence and menopause, when the risk of breast cancer initiation or promotion is relatively high. The major causal hypotheses are reviewed: genetic, viral and endocrine. Although there is an undeniable familial aggregation of breast cancer, the genetic hypothesis suffers since the disease is not transmitted in any recognizable pattern of inheritance. Aggregation is equally consistent with an environmental etiology since family members share many environmental exposures. Although B type RNA virus particles similar to the mouse mammary tumor virus have been found in the milk of women with a family history of breast cancer, only a small proportion of the particles seen in human milk are indistinguishable from the mouse virus and the proportion of women excreting such particles is similar among those with and without a family history of breast cancer. While the role of a virus in breast cancer must be evaluated essentially by laboratory techniques, such studies could be enhanced by use of epidemiologic information in the selection of subjects and materials for evaluation. Among the endocrine hypotheses, those concerning estrogens have been strengthened by a comparison of urinary estrogen profiles of women in populations with different breast cancer risks. (33 references)

6652 AREAS OF RELATIONSHIP BETWEEN IMMUNOLOGY AND CLINICAL ONCOLOGY.

(E.) Southam, C. M. (Jefferson Med. Coll., Philadelphia, Pa.). *Am J Clin Pathol* 62(2):224-242, 1974.

The relationship between immunology and clinical oncology is reviewed with respect to (1) the immune status of cancer patients, (2) effect of immunosuppression on tumor growth, (3) applications of immunology in the detection, diagnosis, and monitoring of cancer, (4) autoimmune reactions to autochthonous cancer, (5) mechanisms of immune reactions against cancer, (6) the potential and problems of immunotherapy, and (7) immunoprophylaxis for cancer. A general description of the immune status of cancer patients (lymphocytic and plasmacytic neoplasms plus other sarcomas and carcinomas) is presented in tabular form. Other tables summarize the effects of methotrexate on the transplantability of a methylcholanthrene-induced sarcoma in syngeneic C57BL/6 mice, tumor incidence in mice treated topically with methylcholanthrene and concurrently with immunosuppressive drugs administered i.p., frequencies of primary neoplasms in patients with genetic or iatrogenic immunodepression, serum α_1-fetoprotein levels in patients with hepatic cancer and other neoplastic and nonneoplastic diseases, serum levels of carcinoembryonic antigen in health and disease, human chorionic gonadotropin levels in choriocarcinoma patients responding to chemotherapy, autoantibodies to cell-surface antigens of melanoma cells, the effect of leukocytes on neuroblastoma cells, the resistance of colonic cancer patients to hematogenously disseminated cancer cells, and the resistance of immunized mice to transplantation of methylcholanthrene-induced sarcomas. (83 references)

6653 CHEMICAL FACTORS (INCLUDING TRACE ELEMENTS) AS ETIOLOGICAL MECHANISMS.

(E.) Harington, J. S. (South African Inst. Med. Res., Johannesburg). In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency For Research on Cancer, 1973, pp. 304-311. (65 references)

6654 NUCLEAR DIFFERENTIATION IN THE DEVELOPMENT OF NORMAL AND NEOPLASTIC TISSUES. *(E.)* Simnett, J. D. (Dep. Pathol., Newcastle upon Tyne, England). In: *Neoplasia and Cell Differentiation.* Basel, Karger, 1974 pp. 1-26. (200 references)

6655 DIFFERENTIATION AND ORGANIZATION IN TERATOMAS. *(E.)* Gaillard, J. A. (Hosp. Ctr., Evreux, France). In: *Neoplasia and Cell Differentiation* Basel, Karger, 1974 pp. 319-349. (100 references)

6656 THE ASSOCIATION OF TUMOR AND EMBRYONIC CELLS *IN VITRO.* *(E.)* Sigot-Luizard, M. F. (Inst. Embryol. Exp. Teratol., CNRS, Nogent sur Marne, France). In: *Neoplasia and Cell Differentiation* Basel, Karger, 1974 pp. 350-379. (72 references)

6657 EMBRYONIC AND TUMOUR CELL INTERACTIONS. *(E.)* Lakshmi, M. S. (Univ. Coll. Hosp. Med. Sch., London, England) and G. V. Sherbet. In: *Neoplasia and Cell Differentiation* Basel, Karger, 1974 pp. 380-399. (64 references)

6658 EXPERIMENTAL TERATOMA. *(E.)* Damjanov, I. (Dep. Pathol., Univ. Zagreb, Yugoslavia) and D. Solter. *Curr Top Pathol* 59:69-130, 1974. (243 references)

6659 COLON CANCER: A DISEASE OF FIBRE DEPLETION OR OF DIETARY EXCESS? *(E.)* Hill, M. J. (Colindale Hosp., England). *Digestion* 11(3/4): 289-306, 1974. (41 references)

6660 THE STATUS OF NUCLEAR DEVELOPMENT: A REVIEW OF DEVELOPMENTS DISCUSSED AT THE 5TH INTERNATIONAL CONGRESS OF RADIATION RESEARCH SEATTLE, U.S.A., JULY, 1974. *(E.)* Brown, B. J. (Sch. Chem., Macquarie Univ., North Ryde, Australia). *At Energy Aust* 17(4):6-13, 1974. (5 references)

6661 THE NATURE OF OCCUPATIONAL CANCER: GENERAL BIOLOGIC CONSIDERATIONS. *(E.)* Dinman, B. D. (Univ. Michigan Sch. Public Health, Ann Arbor). In: *The Nature of Occupational Cancer: A Critical Review of Present Problems.* Illinois, C. C. Thomas, 1974, pp. 3-32. (152 references)

6662 INDUSTRIAL AGENTS ASSOCIATED WITH HUMAN CARCINOGENIC RISK: EVALUATION OF EVIDENCE OF OCCUPATIONAL CARCINOGENESIS. *(E.)* Dinman, B. D. (Univ. Michigan Sch. Public Health, Ann Arbor). In: *The Nature of Occupational Cancer: A Critical Review of Present Problems.* Illinois, C. C. Thomas, 1974, pp. 33-62. (152 references)

6663 TERATOGENIC EFFECTS OF DRUGS: A REVIEW. *(Fin.)* Hirvonen, E. (No affiliation). *Duodecim* 90(20):1392-1400, 1974. (25 references)

6664 PHYSICAL FACTORS AS ETIOLOGICAL MECHANISMS. *(E.)* Timbrell, V. (Llandough Hosp., Penarth, United Kingdom). In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency for Research on Cancer, 1973, pp. 295-303. (35 references)

6665 INFORMATION OBTAINED FROM ANIMAL EXPERIMENTS. *(E.)* Wagner, J. C. (Llandough Hosp., Penarth, United Kingdom) and G. Berry. In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency for Research on Cancer, 1973, pp. 285-288. (19 references)

6666 CANCER IN RELATION TO ENVIRONMENT EXPOSURE. *(E.)* Bohlig, H. (Munic. Hosp., Hamburg, W. Germany) and E. Hain. In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency for Research on Cancer, 1973, pp. 217-221. (30 references)

6667 INVESTIGATIONS USING ANIMALS. *(E.)* Wagner, J. C. (Llandough Hosp., Penarth, United Kingdom) and G. Berry. In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency for Research on Cancer, 1973, pp. 85-88. (14 references)

6668 DRUG-INDUCED BLOOD DYSCRASIAS. *(Fin.)* Palva, I. (No affiliation). *Duodecim* 90(20):1344-1351, 1974. (21 references)

6669 MULTIPLE FACTORS IN CARCINOGENESIS. *(E.)* Epstein, S. (Case West. Reserve Univ., Cleveland, Ohio). *Environ Health Perspect* 9:331-333, 1974. (7 references)

6670 BIOLOGICAL EFFECTS OF INGESTED ASBESTOS: REPORT AND COMMENTARY. *(E.)* Lee, D. H. K. (No affiliation). *Environ Health Perspect* 9:113-122, 1974. (No references)

6671 QUALITATIVE AND QUANTITATIVE DETERMINATION OF CARCINOGENIC HYDROCARBONS (III). *(Jpn.)* Karasawa, Y. (No affiliation). *Aromatikkusu* 26(6): 44-46, 1974. (42 references)

6672 IMMUNOLOGY OF ONCORNAVIRUSES. *(E.)* Hardy, W. D., Jr. (Mem.-Sloan Kettering Cancer Cent., New York, N.Y.). *Vet Clin North Am* 4(1):133-146, 1974. (12 references)

6673 EXPERIMENTAL STOMACH CANCER. *(E.)* Sugimura, T. (No affiliation) and T. Kawachi. *Collect Pap Natl Cancer Cent Res Inst* 9:604-667, 1973. (132 references)

6674 OCCUPATIONAL CANCER IN INTERNAL MEDICINE. *(Ger.)* Berndt, H. (Cent. Inst. Cancer Res., Berlin-Buch, E. Germany). *Internist (Berlin)* 15(8):397-404, 1974. (64 references)

6675 CHEMICAL AGENTS AND CANCER OF THE BLADDER. *(Sw.)* Holmberg, B. (Natl. Board Occup. Safety Health, Stockholm, Sweden). *Lakartidningen* 71(21):2173-2175, 1974. (16 references)

6676 THE CARCINOGEN RISK: A MAJOR ECOLOGICAL PROBLEM. *(Fr.)* Rudali, G. (Radium Inst., Paris, France). *Biomedicine* 20(5):317-321, 1974. (42 references)

6677 REVERSE TRANSCRIPTASE AND ITS POSSIBLE INVOLVEMENT IN TUMOR ETIOLOGY. *(Pol.)* Bartnikova, W. (Inst. Oncol., Gliwice, Poland). *Postepy Hig Med Dosw* 28(3):261-269, 1974. (84 references)

6678 HEMOSTASIS AND CANCER. *(Fr.)* Tobelem, G. M. (Lariboisiere Hosp., Paris, France), L. Israel and J. P. Caen. *Pathol Biol (Paris)* 22(9): 7803-7813, 1974. (75 references)

6679 CURRENT IMMUNOLOGICAL DATA ON HODGKIN'S DISEASE. *(Fr.)* Weisgerber, C. (Res. Inst. Leukem. Blood Dis., Paris, France) and F. M. Kourilsky. *Rev Prat* 24(45):3927-3929, 1974. (5 references)

6680 NATURAL HISTORY OF HODGKIN'S DISEASE. *(Fr.)* Carcassonne, Y. (Dept. Blood Dis., Conception Hosp., Marseille, France), N. Gratecos, J. Foa and R. Favre. *Rev Prat* 24(45):3857-3881, 1974. (4 references)

6681 ELECTRON MICROSCOPY IN THE CYTOLOGICAL CLASSIFICATION OF ACUTE LEUKEMIAS. *(Fr.)* Breton-Gorius, J. (Henri Mondor Hosp., Creteil, France). *Bull Cancer (Paris)* 61(3):367-376, 1974. (30 references)

6682 ACUTE MONOCYTIC LEUKEMIA. *(Fr.)* Lortholary, P. (Franco-Musulman Hosp., Bobigny, France). *Bull Cancer (Paris)* 61(3):327-334, 1974. (22 references)

6683 IMMUNOBIOLOGY OF BURKITT LYMPHOMA AND HUMAN LEUKEMIAS. *(Pol.)* Harlozinska, A. (Dept. Pathol. Anat., Med. Acad., Wroclaw, Poland). *Postepy Hig Med Dosw* 28(3):271-291, 1974. (110 references)

6684 DISCUSSION REMARKS ON A POSSIBLE COMMONALITY IN THE ETIOLOGY OF CHEMICAL CARCINOGENESIS AND ALLERGY. *(E.)* Kaufman, J. J. (Dep. Chem., Johns Hopkins Univ., Baltimore, Md.). *Int J Quantum Chem, Quantum Biol Symp* 1:119-122, 1974. (11 references)

6685 EFFECTS OF CARCINOGENS AND OTHER AGENTS ON HISTONE METHYLATION IN RAT LIVER NUCLEI BY ENDOGENOUS HISTONE LYSINE METHYLTRANSFERASE. *(E.)*

Baxter, C. S. (Coll. Med., Univ. Florida, Gainesville) and P. Byvoet. *Cancer Res* 34(6):1424-1428, 1974.

The effect of carcinogens, especially *S*-adenosyl-L-ethionine (AET), on the methylation of lysine in histone was studied in rat liver nuclei *in vitro* in order to test the hypothesis that carcinogenic agents induce neoplasms by affecting gene suppression through interfering with normal enzyme modification of histones. Methylation of internal lysine residues (determined by column chromatography) in histones was inhibited by AET. The endogenous methyltransferase transferred ethyl groups from AET to histone, but with low efficiency. The histone lysine methyltransferase showed a K_m value of 3.0 μM, and the K_1 value for *S*-adenosylethionine inhibition was 0.17 mM. Product inhibition by *S*-adenosylhomocysteine (K_1 = 12 μM) occurred. Histone methylation in nuclei *in vitro* was sensitive to activated or unstable carcinogens (those derived from 2-aminofluorene and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) but insensitive to inhibition by many carcinogens which require enzymatic activation *in vivo* and to inhibitors of transfer RNA methyltransferases. Repair mechanisms for DNA have been previously shown to exist, whereas none have yet been identified for proteins. Thus, the carcinogenic action of ethionine apparently does not result from modification of macromolecules by electrophilic species, and its effects on histones are permanent.

6686 EFFECT OF CYCLOHEXIMIDE ON PROTEIN AND DNA SYNTHESIS AND ON THE YIELDS OF CHROMOSOMAL ABERRATIONS INDUCED BY DEB AND ENU IN *VICIA FABA*. *(E.)*

Swietlinska, Z. (Inst. Biochem. Biophys., Pol. Acad. Sci., Warsaw), D. Zaborowska and J. Zuk. *Mutat Res* 22:33-38, 1974.

The effect of cycloheximide alone and on aberrations induced by diepoxybutane (DEB) and N-ethyl-N-nitrosourea (ENU) in root tips of *Vicia faba* variety minor (Mazur) was studied. Primary root tips of *Vicia faba* (germinated and grown in moist sand at 24 C) were excised and incubated with cycloheximide, ^{3}H-thymidine or ^{14}C-leucine. Cycloheximide in concentrations higher than 1 μg/ml severely depressed root growth. Leucine incorporation in root tips was inhibited about 80% by 5, 10 and 20 μg/ml cycloheximide and 100% by 50 μg/ml cycloheximide. Starting two hr after the onset of incubation cycloheximide at 1 μg/ml strongly inhibited thymidine incorporation into DNA; almost equal to that produced by adenine at 3000 μg/ml. After a two hr incubation with 1 μg/ml cycloheximide the mitotic index showed a biphasic curve with a very low value at ten hr, a value above controls at 20 hr, another drop to control value at 30 hr and a second rise at 50 hr. Root tips were incubated with DEB ($4 \cdot 10^{-5}$ M) or ENU (10^{-4} M) for two hr and then post-treated for two hr with cycloheximide at 1 μg/ml. Cell damage and aberrations were higher with DEB or ENU alone than with cycloheximide alone. Combination treatment of DEB and cycloheximide gave similar results as with DEB alone. Combination of ENU and cycloheximide depressed the mitotic index at early stages compared with ENU treatment alone. Only a few metaphases were found. At later stages the aberration frequency did not differ from ENU alone. Numbers of experiments are not given. The results indicate that cycloheximide causes the depression of protein synthesis and DNA synthesis in *Vicia faba*. It does not enhance chromosomal aberrations produced by DEB and ENU.

6687 STUDIES ON LIVER MITOCHONDRIAL 5'-ENDONUCLEA ACTIVITY IN RATS FED CARCINOGENIC AMINES AND ON THEIR BINDING TO MITOCHONDRIAL PROTEINS. *(E.)*

Pilon, R. (Cancer Inst., Montreal, Canada) , C. Dupuis and R. Morais. *Chem Biol Interact* 8(6):371-378, 1974.

The effects of 2-acetylaminofluorene (AAF), 2-aminofluorene (AF), and *N*,*N*-dimethyl-4-amino-azobenzene (DAB on the activity of rat liver mitochondrial 5'-endonuclease were studied, as was the amount of carcinogen bound to liver homogenate and mitochondrial fraction proteins. Adult male Wistar rats were maintained for varying periods on diets containing 0.09% AAF, 0.09% AF, or 0.06% DAB. The livers were analyzed for mitochondrial 5'-endonuclease activity and the amount of carcinogen bound to the homogenate and mitochondrial fraction proteins. The enzyme, active against both RNA and DNA, was assayed with poly-A as the substrate. In the concentrations fed, AAF was the most toxic, followed by DAB and AF; hepatomas were detectable after less than 16, 12, and 40 weeks, resp. The activity of mitochondrial 5'-endonuclease was not significantly affected by any of these carcinogens. The amount of AF and AAF bound to the homogenate proteins after four weeks of treatment was about 60-fold higher than that of DAB. The binding of the AF compounds to the mitochondrial fraction proteins was comparatively greater, reaching a level 300-fold higher than that of DAB. The amount of AF residues bound per mg of mitochondrial protein was higher than that of the homogenate, while the reverse was true of DAB. The data suggest no relation between the total amount of these carcinogens bound to the liver cellular proteins *in vivo* and their carcinogenic potential.

6688 ANALYSIS OF 2-ACETYLAMINOFLUORENE: RESIDUES IN LABORATORY CHOW AND MICROBIOLOGICAL MEDIA. *(E.)*

Bowman, M. C. (Natl. Cent. Toxicol. Res., Jefferson, Arkansas) and J. R. King. *Biochem Med* 9(4):390-401, 1974.

Analytical procedures for determining residues of the carcinogen 2-acetylaminofluorene (2-AAF) in laboratory chow and in six different microbiological media by spectrophotofluorescence (SPF) and gas chromatography (GC) are described. The important elements of the SPF method are extraction, a rapid purification on alumina, acid hydrolysis of 2-AAF to 2-aminofluorene (2-AF), further purification by organic solvent extraction of interfering substances from the aqueous amine hydrochloride, conversion of the hydrochloride to the free base (2-AF), and its extraction and final determination as 2-AF by SPF. In the GC method, the extract is purified by solvent partitioning followed by silica gel chromatography with residues being determined directly as 2-AF. In the SPF procedure, recoveries of 2-AF are generally about 10% lower than 2-AAF. Studies with chow and media, containing added 2-AAF and autoclaved, indicated that the compound is stable. The behavior of the hydroxylated metabolites of 2-AAF in the analytical procedure is not known since analytical standards are not available. However, any residues of these compounds extracted by the procedure might be lost during the column absorption step.

6689 RESPONSE AT FIFTEEN WEEKS OF THE BIG BROWN BAT *(EPTESICUS FUSCUS)* TO INTRAPERITONEAL INJECTIONS OF *N*-2-ACETYLAMINOFLUORENE. *(E.)* Heldt, L. V. (Sch. Aerospace Med., Brooks Air Force Base, Tex.), N. H. Rowe and J. D. Spain. *Cancer Res* 34 (10):2807-2809, 1974.

The effect of *N*-2-acetylaminofluorene (AAF), a systemic carcinogen, on the urinary tract of 26 male and female big brown bats was studied following 13 weekly s.c. injections (6 mg each in gum acacia). Urinary tract epithelium from experimental animals sacrificed two wk after the last AAF injection showed hyperplastic changes consisting of nodular hyperplasia in the bladder of 4 of 11 bats and papillary and nodular hyperplasia in the renal pelvis of one bat. No morphologic changes were observed in control animals or in other tissues of experimental bats. The hyperplastic changes most probably represent premalignant changes and are similar to those seen in the urinary tracts of rats given AAF, dibutyl nitrosamine, or *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. The findings are thus consistent with the hypothesis that in the bat, as in the rat, AAF is metabolized to an active carcinogen prior to excretion and that the metabolite then acts on the urinary tract epithelium during excretion. Since the bat is reportedly able to survive body temperature extremes (46-4 C) without apparent adverse effect, it may be a useful model for evaluating the effects of hyper- and hypothermia on cancer biology.

6690 HISTOGENESIS OF PRENEOPLASTIC AND NEOPLASTIC LESIONS OF THE KIDNEY IN RATS INGESTING N-4-(4'-FLUOROBIPHENYL)ACETAMIDE. *(E.)* Reuber, M. D. (No affiliation). *Pathol Microbiol* 41(5):266-274, 1974.

Cellular changes involved in the histogenesis of preneoplastic and neoplastic lesions were studied in the distal convoluted tubules of male and female inbred Buffalo rats ingesting N-4-(4'-fluorobiphenyl)acetamide in the diet. Epithelial cells, lining most of the distal convoluted tubules in the renal cortex, at first increased in size as seen by electron microscopy. Hyperplasia followed, the cells being made up of nuclei with nucleoli, having little cytoplasm (clear or lightly basophilic). The numbers of cells increased until they filled the entire lumen of some tubules. Mitotic figures were not observed. Areas of hyperplasia next developed, where numerically increasing epithelial cells joined together to form discrete lesions. The cells were small, the large nuclei were vesicular and the cytoplasm varied from darkly to lightly basophilic. Hyperplastic nodules formed after several tubules were large enough to form distinct lesions with each basement membrane intact. As the nodules increased in size, there was compression of the adjacent renal parenchyma. Cells in the hyperplastic nodules gradually became atypical, and later appeared malignant, varying in size and shape, with large vesicular nuclei and nucleoli and with sparse cytoplasm. Entire nodules underwent malignant change as individual cells changed. With increasing size, these grew to large, well-developed carcinomas. The cells had darkly or lightly staining cytoplasm and grew in sheets. The tubular pattern had been lost due to breaks in the basement membranes and invasion of adjacent tissues. In some tubules, hyperplastic cells developed degenerative changes (distension by water and/or lipid) and ceased growth. Carcinomas developed cellular coagulation necrosis, particularly in the center. No metastasis was observed. The results of the present study are being compared with the development of renal carcinoma in the human.

6691 A DIPLOID RAT LIVER CELL CULTURE. 1. CHARACTERIZATION AND SENSITIVITY TO AFLATOXIN B_1. *(E.)* Bausher, J. (Coll. Med., Univ. Vermont, Burlington) and W. I. Schaeffer. *In Vitro* 9(4):286-293, 1974.

A method was described for culturing and cloning a diploid epitheloid cell line (tyrosine aminotransferase-synthesizing) from rat liver. The response of this culture and of established cell lines (Chang liver and R-L-T) to aflatoxin B_1 was studied. Rat liver cell lines (RL-NB, RL-PR-C) were derived from three-day-old rats. The diploid culture was more resistant to aflatoxin B_1 (up to 50 μg) than the established cell lines. This was determined by cell counts, residual protein, and the incorporation of [^{14}C] uridine. It is suggested that the resistance of the diploid cell line is the result of *in vitro* acquired properties, or the result of an intrinsic property of a segment of the total cells present in the intact organ. Aflatoxin B_1 is cytotoxic to a variety of cell types but specific for hepatocytes in its carcinogenic properties. It would thus be expected that the target cell would first have to be capable of withstanding the cytotoxic property of aflatoxin. Therefore, this diploid line could represent the target cell population that is susceptible to the carcinogenic action of aflatoxin in the liver.

6692 THE TOXICITY AND MORPHOPATHOLOGICAL EFFECTS OF AFLATOXINS IN TADPOLES. *(Fr.)* Puscariu, F. (Inst. Public Hlth. Med. Res., Cluj, Rumania), V. V. Papillian, M. Gabor and C. Deac. *Arch Roum Pathol Exp Microbiol* 32(2):255-267, 1973.

At 36 hr the LD_{50} of aflatoxin B_1 averaged 1.9 mg/liter for 2-wk-old tadpoles and 2.2 mg/liter for those aged 2-4 wk. At 48 hr the corresponding values were 1.6 and 1.8 mg/liter, resp. The LD_{50} was essentially the same for tadpoles of *Bombina* sp. and *Rana temporaria.* Tadpoles exposed to sublethal doses of aflatoxin G_1 (2-6 µg/ml) developed bulging of the posterior part of the body, which was proportional to the dose of aflatoxin, and had only rudimentary feet. After 5 wk, clear ascites fluid collected in the abdomen. The body volume of these ascitic animals was twice that of controls. *Bombina* sp. was more sensitive to aflatoxin G_1 than *R. temporaria:* ascites developed in the former at a dose of 2 µg/ml, and all of the animals died before developing ascites at a dose of 6 µg/ml. While some of the cells found in ascites fluid were nucleated blood cells, others resembled liver cells in animals which had been given aflatoxins and which later developed cirrhosis. It is suggested that minimum carcinogenic doses of aflatoxins can be established by performing tests on tadpoles.

6693 EARLY CHANGES IN PLOIDY AND CYTOGENETICS OF LIVER CELLS AFTER DIETHYLNITROSAMINE TREATMENT.

(E.) Wrba, H. (No affiliation), P. Fischer, M. Weissberg and L. Wiest. *Recent Results Cancer Res* 44:94-97, 1974.

Changes in the ploidy, cytogenetics, and DNA synthesis of liver cells were observed in newborn Sprague-Dawley rats given diethylnitrosamine (DENA). One application of 10 mg DENA/kg on the day of birth produced a significant rise in the number of tetraploid cells, maintained until the appearance of microcarcinomas on the 30th day, and the loss of binucleate cells usually present during this period. Cells with a ploidy number of eight were shown to appear on the tenth day, 16 on the 25th day, and progressively higher numbers from there on. Doses ranging from 0.1 to 10 mg DENA/kg body weight were shown to produce a dose dependent fall in binucleate liver cells ranging from 40% to between 2 and 4%. The DENA damaged liver (16 mg/kg for three weeks) showed a suppression of DNA synthesis, but no suppression was observed in normal newborns given one application. When given 10 mg DENA/kg s.c. on the 1st, 2nd, 3rd, 7th, 10th, and 12th days after birth and every ten days thereafter, about 50% of the metaphases examined after the third day showed losses in the region of the small metacentric chromosomes. Chromosomes A7 and B5 were often monosomic or completely absent. These changes persisted until the developement of hepatocellular carcinomas.

6694 A DIPLOID RAT LIVER CELL CULTURE. 2. EFFECT OF AFLATOXIN B_1 ON RIBONUCLEIC ACID SYNTHESIS.

(E.) Bausher, J. (Univ. Vermont, Coll. Med., Burlington) and W. I. Schaeffer. *In Vitro* 9(4): 294-301, 1974.

The effect of aflatoxin B_1 on rRNA and total cellular RNA synthesis was studied by sucrose gradient analysis in two rat liver cell cultures. One culture was a diploid line, RL-PR-C, and the other was an established heteroploid line, R-L-T. The RNA synthesis was monitored by using a simultaneous [5-^{3}H]uridine and L-[methyl-^{14}C] methionine label. In both cell lines, rRNA was the type of RNA most affected by treatment with 50 µg per ml of aflatoxin B_1. Synthesis of rRNA in the RL-PR-C line was inhibited by 14%, but the R-L-T line was inhibited by 75% after a two hr incubation with aflatoxin. In the R-L-T culture, the rate of synthesis of rapidly labeled nonmethylated RNA was reduced by 58% while in the diploid cell line the rate of synthesis was stimulated by 75%. This study showed two effects of aflatoxin B_1 on RNA synthesis in rat liver cells grown *in vitro*; one effect was the preferential inhibition of rRNA synthesis, and the other effect was an increase in the rapidly labeled nonmethylated RNA found in the cells of the RL-PR-C cells.

6695 INCREASED PHOSPHORYLATION OF NUCLEAR PHOSPHOPROTEINS IN PRECANCEROUS RATS.

(E.) Ahmed, K. (Toxicol. Res. Lab., VA Hosp., Minneapolis, Minn.). *Res Commun Chem Pathol Pharmacol* 9(4):771-774, 1974.

The early effects of 3'-methyl-4-dimethylaminoazobenzene (3'-methyl DAB) on the phosphorylation of rat liver nuclear phosphoproteins was studied *in vitro.* Male Sprague-Dawley rats were maintained on normal basal diets supplemented with 0.06% 3'-methyl DAB. Their purified liver nuclei were incubated in a reaction medium containing 3 mM ATP (containing trace amounts of (γ-^{32}P)ATP). Total nucleoproteins were precipitated after 60 sec. The liver nuclei from the carcinogen-fed rats showed an increased ability to incorporate ^{32}P from (γ-^{32}P)-ATP into endogenous phosphoproteins. The largest increase in the rate of phosphorylation of the nuclear proteins was seen in the livers of animals fed the carcinogenic diet for 26 days; this period represents the early hyperplastic response of the tissue to 3'-methyl DAB. The results suggest that the increase in phosphorylation of the nuclear phosphoproteins occurred in response to a carcinogenic stimulus in the tissue as an early event in the precancerous state. The response may be a consequence of increased sites available for phosporylation or increased activity of the endogenous phosphokinases in the nucleus.

6696 PARTIAL INHIBITION OF HYDROXYUREA ON LIVER TUMORS INDUCED BY N,N-DIMETHYL-4-AMINOAZOBENZENE.

(E.) Lin, J. K. (Coll. Med., Natl. Taiwan Univ., Taipei) and I. S. Chiu. *J Formosan Med Assoc* 73(1):220-226, 1974.

The effect of hydroxyurea on the propagation of N,N-dimethyl-4-aminoazobenzene (DAB)-induced primary liver tumors was studied *in vivo.* Male Sprague-Dawley rats were fed a basal diet containing 0.06% DAB. After 70 days, they were put on a normal diet, and five days later some were given 10 mg hydroxyurea i.p.; the injections were repeated every other day for 159 days. The hydroxyurea-treated and control rats did not differ in total survival rate, but the average body weight was higher in the hydroxyurea-treated rats and hepatomegaly was more common in the controls. In addition, longevity calculated from the cessation of DAB treatment was greater in the hydroxyurea-treated animals. While seven of eight control rats developed primary liver carcinomas, only three of eight hydroxyurea-treated rats developed primary liver tumors; further, while two control rats developed lung metastases, none of the hydroxyurea-treated rats developed any. Thus, hydroxyurea appears to have a partial inhibitory effect on the further development of liver carcinomas in DAB-fed rats.

6697 MICROSOME-DEPENDENT BINDING OF BENZO(a) PYRENE AND AFLATOXIN B_1 TO DNA AND BENZO (a)PYRENE BINDING TO AFLATOXIN-CONJUGATED DNA. *(E.)*

Alexandrov, K. (Inst. Sci. Res. Cancer, Villejuif, France) and C. Frayssinet. *Cancer Res* 34(12):3289-3295, 1974.

Aflatoxin B_1-conjugated DNA and benzo(a)pyrene (BP)-conjugated DNA were isolated after incubation *in vitro* with liver microsomes from rats treated with phenobarbital (PB), methylcholanthrene (MC), or phenobarbital plus methylcholanthrene. A DNA complex with both carcinogens was obtained by fixation of BP to the aflatoxin B_1-conjugated DNA. Phenobarbital was injected i.p. into male Wistar rats (Commentry strain) at 80 mg/kg for three consecutive days. MC (40 mg/kg) was injected i.p. 48 hr before sacrifice. The microsomes were isolated and incubated with 5 mg DNA and the NADPH-generating system. In two other instances, 80 nM of BP-^{3}H or 0.5 μM aflatoxin B_1-^{14}C was added to the above. The UV absorption spectra of aflatoxin B_1-^{14}C-conjugated DNA was determined from 320-400 nM. The BP-^{3}H binding to DNA was maximum with MC and PB + MC microsomes. Fixation of BP-^{3}H metabolites to DNA without microsomes was similar to that found with MC microsomes. A non-labeled BP-conjugated DNA with ^{3}H-labeled BP metabolites indicated that maximum fixation to specific DNA sites occurred with the initial BP metabolite addition. Absorption for the aflatoxin B_1-^{14}C-conjugated DNA was always maximum at 360-370 nm. Radioactivity measurements gave a maximum with the MC microsomes. The number of nucleotides bound by aflatoxin B_1 decreased in the presence of all treated microsomes. 7,8-Benzoflavone caused an 80% inhibition of BP binding to DNA with MC microsomes and PB + MC microsomes, but only 20 and 0%, resp., with aflatoxin B_1. With control and PB microsomes, a 30-50% increase in the binding level of aflatoxin B_1 to DNA was obtained, while with BP the binding decreased 32% with these microsomes. The results suggest that the binding species of the acetate-soluble BP metabolites are relatively stable. MC activity is more essential to the microsome-dependent binding of BP to DNA than for the binding of aflatoxin B_1 to DNA. This is in agreement with previous studies.

6698 ASPECTS OF EXPERIMENTAL HEPATOCARCINOGENESIS. PART IV. CHANGES IN THE LIVER FOLLOWING CESSATION OF CARCINOGEN ADMINISTRATION. *(E.)*

Timme, A. H. (Dep. Pathol., Univ. Cape Town, South Africa). *S Afr Med J* 48(31):1331-1334, 1974.

In order to separate the non-specific toxic effects of a chemical from its possible carcinogenic effects, the substance was administered for a certain period, following which the animals were returned to normal conditions and observed for irreversible changes over a relatively long period of time. Thirty male rats, weighing 100 g at the start of the experiment, were fed 0.05% by weight of p-dimethylaminoazobenzene (p-DAB) in maize for 20 weeks and then returned to the normal laboratory diet. Fifteen animals developed tumors within two months of cessation of administration of the carcinogen. In addition to trabecular carcinomas, adenocarcinomas and mixed tumors, severe cirrhotic changes were also noted. Large liver tumors were found in two of the 15 survivors which died at three months and six which died at five months. The remaining seven animals, killed six months after the feeding of p-DAB was stopped, showed no liver tumors. The cirrhotic changes regressed almost completely, so that the livers of animals which survived at least five months showed minimal granular changes. Nucleolar hypertrophy, abnormalities of the mitochondria, and an increase in the smooth endoplasmic reticulum were observed. The amount of glycogen was decreased, even in the livers of animals which had survived six months and glycogen was present in only about half of the cells of the liver biopsy specimens of the animals which died three months after cessation of feeding of p-DAB. It is suggested that these findings may have a bearing on the occurrence of primary liver cancer in non-cirrhotic patients since hepatocellular injury may regress with time before the development of the carcinoma.

6699 RESPONSE OF CELL CULTURES TO ASBESTOS FIBERS. *(E.)*

Bruch, J. (Univ. Dusseldorf, Germany). *Environ Health Perspect* 9:253-254, 1974.

To determine whether asbestos fibers have a cytopathogenic effect, the reaction of macrophages and of line L929 cells to crysotile fibers and milled crysotile was studied *in vitro* during successive phases of particle incorporation. Comparable tests were made with glass fibers and milled glass. Morphological studies by phase contrast and interference contrast microscopy showed that many fibers are incorporated by both cell types and that long fibers may be simultaneously phagocytosed by several cells. Intracellular localization of the fibers was indicated by the direct attachment of lysosomes to the fibers. Through electron microscopy it was seen that successive stages of phagocytosis can be present simultaneously along a single fiber. With long fibers, increases in concentration of lactic dehydrogenase (LDH) and in percentage of erythrosin B-stained cells were observed, compared with controls, indicating abnormal membrane permeability. At the same time, lactate production was also elevated over controls, indicating continuing viability of the culture. With milled fibers, concentrations of LDH and percentage of erythrosin B-stained cells differed from those with long fibers, and were generally more similar to those of the controls. The high lactate production and elevated cell membrane permeability after the initial phagocytic phase persisted up to three months after a single exposure to long fibers, indicating that the size and shape of the fibers are essential factors contributing to the pathogenicity of asbestos and are more important than physiochemical properties of the surfaces. Thus, long fibers seem to produce a protracted pathogenic effect without an acute, cytotoxic effect.

6700 A MICROSOMAL MEMBRANE ALTERATION FOLLOWING ETHIONINE INTOXICATION. *(E.)*

Kisilevsky, R. (Dep. Pathol., Queen's Univ., Kingston, Canada) and L. Weiler. *Cancer Res* 34(12):3421-3427, 1974.

The effects of prolonged periods of ethionine intoxication on the liver microsomal membranes of female Sprague-Dawley rats were studied. The animals were injected i.p. with 1 mg/g of D,L-ethionine and sacrificed 4, 10, 16, or 24 hr later. The binding of the liver ribosomes to the microsomal membranes was studied *via* three different incubations: (a) incubation of membranes with or without ribosomes; (b) reactivation of inactivated RNase in the presence of microsomal membranes obtained from the first incubation; and (c) determination of the RNase activity during the reactivation of RNase. At both four and 24 hr after ethionine administration, there was a significant reduction in the ratio of RNA to protein in the liver microsomes. Based on the disulfide-interchange activity of the microsomal membranes, it was shown that control membranes (from untreated rats) bound control ribosomes but did not bind ribosomes from ethionine-treated animals. Membranes taken after four hr from ethionine-treated rats would bind control ribosomes but not ribosomes taken four hr after ethionine treatment, and membranes taken 24 hr after ethionine treatment would not bind control ribosomes or ribosomes taken from ethionine-treated animals. The data indicate that separation of the ribosomes from the endoplasmic reticulum four hr after the administration of ethionine is due to a ribosome lesion and not a membrane lesion. Membrane lesions occurred later, so that 24 hr after ethionine intoxication, both ribosomal and membrane lesions are present.

6701 LOCALIZATION OF THE LABELLED 5-AZACYTIDINE IN CULTURED MOUSE EMBRYONIC CELLS. *(E.)*

Seifertova, M. (Inst. Org. Chem. Biochem., Czechoslovak Acad. Sci., Prague), J. Vesely and A. Cihak. *Experientia* 30(12):1463-1465, 1974.

The uptake of the pyrimidine analogue 5-azacytidine was studied and its localization over heterochromatin was observed in fetal mouse fibroblasts *in vitro*. Primary cultures were prepared from 13-day-old mouse embryos, and grown in Roux bottles (1 x 10^6 cells/ml). After 18 hr of cultivation at 37 C, 10^{-5} M (0.2 μCi/ml) 5-azacytidine-4-^{14}C (33.7 mCi/mM) was added; 1 hr later the radioactive medium was removed and a fresh medium added. The cells were harvested at 2-hr intervals following 1-hr exposure to colchicine (0.05 μg/ml). After fixation and a 2-month development in stripping film the cells were treated with Giemsa stain in a phosphate buffer and photographed. In each instance 25-30 individual karyotypes were counted. The number and size of nucleoli varied considerably during the experiment; the largest were found 8 hr after removal of the labeled analogue. At all recovery times the label was concentrated close to the nucleoli or to the nuclear membrane, and was most evident at 6 hr. The tendency observed in controls for the centromeric (proximal) heterochromatin to aggregate into star-shaped clusters was much more pronounced following 5-azacytidine-4, especially at 4-6 hr. Thread-like extensions of centromeres associated in some cases with decondensed heterochromatin were clearly noticeable. At 6-24 hr achromatic gaps, elongated chromatids and fragmented chromosomes were seen. The highest percentage of labeled chromosomes was seen at 10 hr. Except at 16-22 hr, grains were more frequently observed over heterochromatin. At 16 hr the label predominated over the euchromatin (distal) region, and from 16-22 hr mitoses diminished in number. The presence of large numbers of pyknotic cells was observed at various time intervals. Mitotic figures with chromosomes labeled prevalently over heterochromatin began to reappear at 22-24 hr; the percentage of labeled chromosomes remained relatively high. Cell division was evidently delayed or even blocked. The data suggest that the cells which took up the analogue at the drug-sensitive period of their generation cell cycle underwent pyknotic degeneration. It is concluded that the lethal action of 5-azacytidine-4 in the synapsis phase, the localization of the labeled analogue in the nuclear and chromosomal heterochromatin, as well as previous findings, indicate that the drug may be of value in the investigation of nucleolar function and its relation to heterochromatin.

6702 BROMODEOXYURIDINE DEPENDENCE -- CHARACTERIZATION OF THE MUTANT CELLS. *(E.)*

Davidson, R. L. (Harvard Med. Sch., Boston, Mass.) and M. D. Bick. *Cold Spring Harbor Conf Cell Proliferation* 1:205-212, 1974.

Since 5-bromodeoxyuridine (BrdU) is generally toxic to all cells except those which do not incorporate it, the properties of the BrdU-dependent cells were examined to determine if there were alterations in the growth process. A pigment clone (W1), isolated from the Syrian hamster melanoma line RPM1 3460, was found to be even more sensitive to BrdU than the uncloned line. After exposure to 10^{-5} M BrdU medium (frequency of cell survival was 1 x 10^{-4}) the cells were then passed every 2-3 weeks to new BrdU medium. Large colonies (B1, B2, B3, etc.) of unpigmented, rapidly growing cells were found to have high levels of thymidine kinase activity and about twice as many chromosomes as the parent melanoma cells. In BrdU absence, the BrdU-resistant cells were observed to require BrdU for maximum growth. The BrdU-dependent cell populations were able to multiply slowly for at least a few generations, indicating that the rate of growth was related to the BrdU concentration. In the absence of BrdU, the B4 cells were much more flattened and spread out (a reaction opposite of BrdU-sensitive cells). Using media such as thymidine to study the specificity of the requirement of BrdU, B4 cell growth was inhibited due to competition of thymidine with BrdU, suggesting that there is incorporation of BrdU into the DNA of dependent cells. There was found to be an increase (0.06 g/ml) in buoyant density of the dependent cells, indicating a significant replacement of thymine by bromouracil (BrUra) in the DNA of the dependent cells. Results of labeling and irradiation experiments suggested that the BrdU was evenly distributed throughout the DNA. Exposure to a medium containing aminopterin plus BrdU almost completely inhibited growth of the cells. A line of cells (HAB) did grow and its DNA buoyant density suggested that about 10% of the thymine residues in the DNA were not replaced by BrUra. Experiments with HAB-B cells showed similar results with respect to BrdU dependency as did the B4 studies. The authors suggest two possible alternatives: 1) BrdU acts as a mutagen, a suppressor of differentiated functions or as an inducer of latent viruses, and 2) mutation in a DNA polymerase occurs so that the enzyme functions only with BrdU, and not with thymidine.

6703 THE DETAILED CHROMOSOME CONSTITUTION OF A BENZYPYRENE-INDUCED RAT SARCOMA. A TENTATIVE MODEL FOR G-BAND ANALYSIS IN SOLID TUMORS.

(E.) Levan, G. (Inst. Genet., Univ. Lund, Sweden). *Hereditas* 78(2):273-290, 1974.

Tumors induced in Wistar/Furth rats by s.c. injection of 3,4-benzpyrene (BP) (0.1 mg), were cultured and the chromosome content was compared with that of the actual tumor. G-band analysis was applied to the culture. The tumor (an anaplastic fibrosarcoma) was harvested for chromosome preparation and culture. Of the fixed cells 95.8% were diploid and 4.2% were tetraploid while 77.8% of the culture cells were diploid, 21.8% were tetraploid and 0.6% were octoploid. The mean chromosome number in the diploid region was 41.6 for the fixed cells and 42.2 for the culture cells. Of 58 cells karyotyped, 55 were in the diploid region. No cells were found with normal rat karyotype and no two karyotypes were identical. The tumor cells were characterized by losses of chromosomes A1, A3, C12 and of B and D groups. The losses were compensated for by gains of chromosomal markers. Of 59 culture cells karyotyped, 34 were studied by G-banding and 25 by conventional orcein staining. In both methods the same chromosomes were involved in losses. The 25 unbanded cells belonged to 24 karyotypes while the 34 G-banded cells had 32 karyotypes. Chromosomes B4, D14 or D15 were usually lost. Mar 16 and mar 22 were present in most cells. A total of 32 markers were observed in 34 cells and most only occurred in single cells, suggesting that new markers are formed continuously. The average genome length of tumor cells was 15.8 units more than chromosomes of a normal diploid cell. In the loss of A1 chromosomes, compensation was often double. No cell contained less than the normal A2 material. Loss of chromosome B4 was compensated by gain of a marker containing most of the B4 material. C13 and the D group were never involved in marker formation. In early tumor development mar 22 and mar 13 appeared. Next came mar 16, then mar 23, then mar 2. It was suggested that the stem line itself carries heritable factors for chromosome variations linked closely to important viability factors. G-banding gave satisfactory results.

6704 EFFECTS OF INDUCERS AND EPOXIDE HYDRASE ON THE METABOLISM OF BENZO[*a*]PYRENE BY LIVER MICROSOMES AND A RECONSTITUTED SYSTEM: ANALYSIS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY.

(E.) Holder, G. (Natl. Inst. Arthritis and Metab. Dis., Natl. Inst. Health, Bethesda, Md.), H. Yagi, P. Dansette, D. M. Jerina, W. Levin, A. Y. H. Lu and A. H. Conney. *Proc Natl Acad Sci USA* 71(11):4356-4360, 1974.

The mobilities of 24 potential metabolites of benzo-[*a*]pyrene (BP) were studied using high pressure liquid chromatography; in addition, the effects of phenobarbital- and 3-methylcholanthrene-induction on the profile of BP metabolites formed by rat liver microsomes were investigated. The effects of epoxide hydrase (EC 4.2.1.63) on the metabolism of BP by a partially purified mixed-function oxidase system was also evaluated. The 24 metabolites included 12 phenols, 5 quinones, 4 dihydrodiols, and 3 oxides, and the chromatographic procedure separated and quantified the metabolites into three groups: phenols, quinones, and dihydrodiols. Phenobarbital or 3-methylcholanthrene enhanced the metabolism of BP by liver microsomes and altered the relative amounts of the metabolites formed. In the absence of epoxide hydrase, BP was metabolized primarily to phenols and quinones but was not appreciably metabolized to dihydrodiols by a solubilized, reconstituted cytochrome P-448 monooxygenase system. Addition of epoxide hydrase resulted in the formation of BP dihydrodiols with a concomitant decrease in the formation of phenolic metabolites. This suggests that BP undergoes metabolism via arene oxides that are precursors for dihydrodiols and phenols.

6705 STUDY OF THE POSSIBLE ADSORPTION OF BENZO (a)PYRENE AND ITS CONTENT IN SOME SOVIET BRANDS OF COMMERCIAL CARBON BLACK.

(Rus.) Pylev, L. M. (Inst. Exp. Clin. Oncol., Moscow, USSR) and G. D. Iankova. *Gig Tr Prof Zabol* (4):52-53, 1974.

Studies were carried out on channel black (cb) and thermal carbon black with the particle sizes of 12.8 nm and 304 nm; and specific surface areas of 250 m^2/g and 10.6 m^2/g, resp. Benzo(a)pyrene (BP) was determined spectrophotometrically on benzene extracts from 10 g samples of each type of carbon black. No BP was detected in channel carbon black extracts, while thermal carbon black contained an average of 32,800 µg/kg. When exposed to a current of BP at 900 C for 90 min, channel carbon black adsorbed three to nine times more BP than the thermal brand. It is suggested that very fine carbon blacks' can adsorb BP from other sources, thus enhancing their carcinogenicity.

6706 THE EFFECT OF 3,4-BENZOPYRENE ON ATPase ACTIVITY OF DIFFERENTIATING MYOGENIC CULTURE CELLS.

(Rus.) Bundiuk, L. S. (Inst. Oncol. Problems, Kiev, USSR) and V. A. Shuklinov. *Tsitologiia* 16(10): 1309-1311, 1974.

Cells from connective and muscular tissues of 1-2-day-old rats were cultured in medium 199 with 10% bovine serum. Benzopyrene (BP) (0.1 and 3.0 μg/ml) was added 24 hr after plating the cells in culture. The cultures were stained 2-144 hr after BP administration. A correlation was observed between the degree of cell differentiation and inhibition of Mg-dependent ATPase activity. No BP effect was observed on Na-activated ATPase activity which tended to increase in myoblasts and multinucleated coenocytes. Ca-activated ATPase did not change in poorly differentiated cells, but increased in myoblasts and coenocytes. The results of the experiments indicate that the disruption of differentiation of myogenic cells affected by BP was related to impaired myosin synthesis. It is suggested that the inhibition of myogenic cell differentiation resulted from the changes in the cellular energy metabolism and decreased mobility which affected the formation of polynucleated coenocytes.

6707 THE ROLE OF ARYL HYDROCARBON HYDROXYLASE IN SKIN TUMOR INITIATION BY 7,12-DIMETHYLBENZ(a)ANTHRACENE AND 1,2,5,6-DIBENZANTHRACENE USING DNA BINDING AND THYMIDINE-^{3}H INCORPORATION INTO DNA AS CRITERIA. *(E.)* Bowden, G. T. (Univ. Wisconsin Med. Sch., Madison), T. J. Slaga, B. G. Shapas and R. K. Boutwell. *Cancer Res* 34(10):2634-2642, 1974.

The effects of 5,6-benzoflavone (5,6-BF) and 7,8-benzoflavone (7,8-BF) on epidermal aryl hydrocarbon hydroxylase (AHH) activity and DNA synthesis were studied in female CD-1 mice treated topically with 1,2,5,6-dibenzanthracene (DBA) or 7,11-dimethylbenz(a)anthracene (DMBA). 7,8-BF (3.67 μM/mouse) caused a 50-94% decrease in skin tumor formation in mice given DMBA (3.9, 39, or 200 mM) at the same time followed by promotion with croton oil or 12-*O*-tetradecanoyl-phorbol-13-acetate. The effect was inversely related to the DMBA dose. 5,6-BF (3.67 μM) produced about a 30%, and phenobarbital (4.31 μM) about a 20% inhibition of DMBA-induced tumor formation which was independent of the DMBA dose. 7,8-BF and phenobarbital produced a 100% increase and a 50% decrease, resp., in tumor incidence in mice treated with a high DBA dose (180 nM). Phenobarbital produced a 65% inhibition of and 7,8-BF had no effect on tumor initiation in mice given a low DBA dose (36 nM). 5,6-BF had no effect at either dose. When binding of ^{3}H-DMBA or ^{3}H-DBA to epidermal DNA, RNA, and protein was studied in 5,6-BF, 7,8-BF, and phenobarbital-treated mice, a direct correlation was found between the extent of macromolecular binding and tumorigenic ability for DMBA but not for DBA. DBA (180 nM) proved to be a stronger inducer of epidermal AHH or ^{3}H-DBA activity (1200% after 12 hr) than either DMBA (600%) or 5,6-BF (350%). Neither 7,8-BF nor phenobarbital was able to induce AHH. The DMBA-induced inhibition of ^{3}H-thymidine incorporation into epidermal DNA was partially blocked by simultaneous treatment with 7,8-BF, 5,6-BF and phenobarbital; the degree of the blocking was correlated with the ability of the three compounds to affect macromolecular binding of ^{3}H-DMBA. These results indicate that: 1) 7,8-BF inhibits enzymes involved in the activation of DMBA to its carcinogenic form to a greater extent than it inhibits enzymes involved in DMBA detoxification; 2) 7,8-BF may inhibit DBA detoxifying enzymes relatively more than its activating enzymes; 3) DBA but not DMBA may have a specific phenobarbital-inducible detoxification system in mouse epidermis.

6708 DEPENDENCE OF SPECIFIC METABOLISM OF BENZO(A)PYRENE ON THE INDUCER OF HYDROXYLASE ACTIVITY. *(E.)* Rasmussen, R. E. (Cancer Res. Inst., U. California, San Francisco) and I. Y. Wang. *Cancer Res* 34(9):2290-2295, 1974.

Adult male Sprague-Dawley rats were treated with polycyclic hydrocarbons, phenobarbital (PB), or barbital; and the induced hydroxylase enzymes in liver and lung were studied in terms of the specific metabolites of benzo(*a*)pyrene (BP) that were produced in an *in vitro* incubation system. The metabolite profile produced by phenobarbital- or barbital-induced enzymes differed from that found with the hydrocarbon-induced liver enzymes in that only the production of 4,5-dihydro-4,5-dihydroxy-BP and 3-hydroxy-BP was increased over the control level, while the production of all identified metabolites was increased when the hydrocarbons were used as inducers. An exception was benzo(*a*)pyrene, which was essentially inactive as an inducer for either liver or lung enzymes. PB and barbital did not increase enzyme activity in the lung, but BP, 3-methylcholanthrene (3-MC), and 1,2-benzanthracene did. When the induced liver enzymes were tested for their ability to activate BP to a bacterial mutagen, the PB-induced enzymes were much more active than the 3-MC-induced enzymes when low levels (less than 1 mg/ml) of enzyme protein were present in the incubation mixtures. At higher levels of enzyme protein, the 3-MC-induced enzymes were more active. Addition of an epoxide hydrase inhibitor, 1,2,epoxy-3,3,3,-trichloropropane, to the test mixtures with PB-induced enzymes greatly increased the mutagenic activity but only slightly increased the activity obtained with the 3-MC-induced enzymes. Thus, PB and polycyclic hydrocarbons appear to induce enzymes in rat liver with different specificities toward BP, the induced enzymes activated BP to a form that was mutagenic for bacteria, and inhibition of the epoxide hydrase induced by PB (probably the 4,5-epoxide) allowed the accumulation of a form of BP that was mutagenic for bacteria.

6709 LYSOSOMAL PHYSIOLOGY IN *TETRAHYMENA*. III. PHARMACOLOGICAL STUDIES ON ACID HYDROLASE RELEASE AND THE INGESTION AND EGESTION OF DIMETHYLBENZANTHRACENE PARTICLES. *(E.)* Rothstein, T. L. (Duke Univ. Med. Cent., Durham, N. C.) and J. J. Blum. *J Cell Biol* 62(3):844-859, 1974.

The effects of various pharmacologic agents on the ability of logarithmically growing cultures of *Tetrahymena* to ingest ^{14}C-labeled 9,10-dimethyl-1,2-benzanthracene particles (DMBA, 0.1 μm diameter), to egest preingested particles, and to release acid phosphatase, ribonuclease, and α-glucosidase were studied. Serotonin (0.39 mM), caffeine (0.10 mM) and dibutyryl cyclic AMP (1.0 mM) increased DMBA particle ingestion by 41%, 70% and 42%, resp. after 15 min, but did not alter the rate of release of any of the hydrolases. Catecholamines had no effect on either particle ingestion or acid hydrolase release. Particle ingestion at 15 min, however, was inhibited from 20-70% by the catecholamine antagonists dichloroisoproterenol (0.10 mM), desmethylimipramine (0.071 mM), reserpine (0.029 mM) and phenoxybenzamine (0.20 mM); these same agents increased release of acid phosphatase and ribonuclease by 60-275% during a 5-hr incubation. Desmethylimipramine caused increased release of hydrolases within 1 hr and also increased the energy of activation for the release of acid phosphatase. Dichloroisoproterenol, however, caused a slower release of hydrolases which was secondary to increased cellular content. Both these drugs enhanced the egestion of preingested DMBA particles *via* the cytoproct of *Tetrahymena*, supporting the view that acid hydrolase release occurs through this type of mechanism. Colchicine (1000 μg/ml), vinblastine (20 μg/ml) and cytochalasin B (12 μg/ml) caused a 20-50% inhibition of DMBA particle ingestion but had no effect on acid hydrolase release. It is concluded that both microtubules and microfilaments play a role in the ingestion process of *Tetrahymena* and that this process may be controlled in part by a cyclic AMP-mediated serotoninergic and andrenergic system.

6710 CONCENTRATION CHANGES OF FREE RADICALS IN THE TISSUES OF RATS AFTER THE INJECTION OF DMBA AND ITS NON-CARCINOGENIC ANALOGUE. *(Rus.)* Petiaev, M. M. (Rostov Sci. Res. Oncol. Inst., U.S.S.R.), V. S. Skotnikov and L. V. Taradin. *Biofizika* 19(1):191-193, 1974.

Electron paramagnetic resonance studies were carried out on the viscera tissues of albino, random-bred, 120-150 g rats after a single injection of 2 mg of 7,12-dimethylbenz(a)anthracene (DMBA). Anthracene was used as a non-carcinogenic analogue. The kinetic curves of concentration changes of paramagnetic centers in the tissues of the liver, lungs, brain, spleen, adrenal glands and thymus showed wave-like fluctuations. Significant differences were observed in the curves for the spleen and thymus, and, particularly, the liver and brain. The data obtained suggest a special bioantioxydating function of the liver, which exhibited a greater response to the action of the carcinogen than to its non-carcinogenic analogue. It is pointed out that no specific action of the carcinogen was observed at the early stages of carcinogenesis.

6711 DISTURBANCE OF THE IMMUNE-SURVEILLANCE MECHANISM IN MICE DURING CHEMICAL CARCINOGENESIS. *(E.)* Matsuoka, Y. (Osaka Univ. Med. Sch., Japan), U. Yamashita, H. Fujiwara, H. Senoh and M. Kitagawa. *Gann Monogr Cancer Res* 16:3-14, 1974.

The cellular mechanism involved in the immunosuppression induced in mice by 7,12-dimethylbenz(a)-anthracene (DMBA) was analyzed. When administered simultaneously with antigen, DMBA in carcinogenic doses (1.0 mg) produced no immunosuppression and even occasionally augmented the serum antibody titers in C3H/He mice. The development of immunological memory in these animals was persistently suppressed by the drug. The development of antibody-forming cells from memory cells was also depressed by DMBA, but this effect was transient. The cellular mechanism of DMBA-induced immunosuppression was further analyzed in terms of T and B cells using hapten-protein conjugates as antigens. The development of both carrier-primed helper cells and antibody-forming cell precursors was markedly depressed by DMBA. The depression of T-cell activity (helper acticity) seemed to be primary for DMBA-induced suppression, because the depressed B cell activity could be restored by supplementation of the helper cells. When normal syngeneic thymocytes were administered i.v. to DMBA-treated mice, most of the treated animals developed a variety of wasting symptoms resembling those of runt disease or graft-versus-host reaction and many of them died within 4-5 weeks. Histological examination showed a loss of folicular organization in the spleen and lymph nodes, a similar, but less marked, change being observed in mice given DMBA alone. The results suggest that the immune-surveillance mechanism in these mice was severely disturbed not only by the direct action of DMBA but also by the secondary reaction of immunocompetent cells to self-constituents under specific conditions induced by DMBA.

6712 MAMMARY NEOPLASIA IN SPRAGUE-DAWLEY OR LONG-EVANS RATS AFTER *IN VITRO* 7,12-DIMETHYLBENZ(α)ANTHRACENE. *(E.)* Brown, R. D. (Brookhaven Natl. Lab. Assoc. Univ., Inc., Upton, Long Island, N.Y.) and D. J. Shellabarger. *Cancer Res* 34(10):2594-2595, 1974.

The development of mammary tumors was compared in female Sprague-Dawley and Long-Evans rats in which mammary tissue was excised, exposed *in vitro* to 7,12-dimethylbenz(a)anthracene (DMBA, 30 mg/10 ml for 10 min) and then grafted onto the backs of the same animals. Other rats were given 20 mg DMBA by stomach tube. Long-Evans rats showed a smaller mammary neoplastic response than Sprague-Dawley rats with respect to tumor incidences (6 of 48 *vs* 18 of 47 transplants after 5 months). Histologically, the tumors which developed in the Long-Evans rats were equally divided among adenocarcinoma, fibroadenoma, and sarcoma while those in Sprague-Dawley rats were equally divided between adenocarcinoma and fibroadenoma with no sarcomas being observed. No Long-Evans controls and only one Sprague-Dawley control which received sham transplants developed a mammary neoplasm (fibroadenoma). DMBA given by gastric intubation also produced a lower mammary neoplastic response in Long-Evans than in Sprague-Dawley rats: 11 of 16 Sprague-Dawley rats developed a total of 29 mammary adenocarcinomas by 5 months after carcinogen administration, whereas 8 of 27 Long-Evans females developed 12 adenocarcinomas. It is suggested that the mechanism responsible for the greater mammary neoplastic response to DMBA in Sprague-Dawley rats does not reside in the mammary tissue itself but rather depends on strain differences that act after the initial DMBA-mammary tissue interaction. The strain difference may be at the level of the ovarian-pituitary-hypothalamic axis.

713 CYTOCHEMICAL DETERMINATION OF ACID DNASE ACTIVITY IN LYMPHOCYTE NUCLEI OF BALB/c MICE
ITH METHYLCHOLANTHRENE-INDUCED TUMORS. *(Rus.)* Oudim-
evkovitch, K. A. (Inst. Oncol. Probl., Acad. Sci.,
iev, U.S.S.R.), D. F. Gluzman and Y. A. Umansky.
sitologiia 16(11):1392-1396, 1974.

ocalization and activity of acid DNAse in lympho-
ytes of different groups of lymphatic nodes in BALB/c
ice with methylcholanthrene-induced tumors was in-
estigated. Into the muscle of the right hind leg of
.5-2-month-old male BALB/c mice, 0.1 mg of methyl-
holanthrene in 0.1 ml of peach oil was introduced.
hen sarcomas developed (4-4.5 months later), the
ancerous mice were killed and the lymph nodes were
emoved for analysis. Experimental evidence showed
hat the activity of acid DNAse in the nuclei of
ymphocytes of cancerous mice was significantly
igher than that in nuclei of control animals. The
ncrease in activity of acid DNAse was accompanied
y an increase in DNA synthesis.

714 FATTY ACID SYNTHETASE IN CHEMICALLY INDUCED MAMMARY CARCINOMAS.
(E.) Schroering, J.
. (Univ. Kentucky Med. Cent., Lexington) and E. D.
ees. *Res Commun Chem Pathol Pharmacol* 9(4):775-778,
974.

ne nature of the enzyme, fatty acid synthetase, in
nemically induced mammary carcinomas was studied in
emale Sprague-Dawley rats. The mammary tumors were
nduced by intragastric administration of 3-methyl-
nolanthrene. Purified high speed supernatants from
ooled tumors and pooled normal mammary glands were
ssayed for fatty acid synthetase activity based on
ne incorporation of acetyl CoA into fatty acids.
ne elution pattern of the tumor supernatant on
ephadex columns was similar to that of the mammary
and supernatant. The mammary tumor and mammary
and fatty acid synthetases were also similar in
erms of their kinetic behavior, pH optimum, and lack
inhibition by 0.16 mM malonyl CoA. Thus, the re-
ilts indicate that the fatty acid synthetase from
it mammary carcinomas is structurally and functional-
similar to the enzyme obtained from normal mammary
and.

715 EFFECT OF THE ANTIESTROGEN NAFOXIDINE ON THE OCCURRENCE OF ESTROGEN-DEPENDENT RENAL
IMORS IN HAMSTER. *(E.)* Antonio, P. (Fac. Med.,
lencia, Spain), M. Gabaldón, T. Lacomba and A. Juan.
rm Metab Res 6(6):522-524, 1974.

ie effect of the simultaneous administration of di-
hylstilbestrol (DES) and nafoxidine upon the hepatic
ycuronyl transferase activity in golden Syrian ham-
ers was investigated. The animals were divided into
ur groups and received the following doses, s.c.:
2 ml of a 0.30% suspension of DES three times/week;
2 ml of a 0.30% suspension of DES three times/week
d 0.2 ml of a 0.50% suspension of nafoxidine two
mes/week, only the DES being administered after
e sixth month of treatment; 0.4 ml of a 2.44% sus-
nsion of polydiethylstilbestrol phosphate (PSP)
nthly during the first five months and every two
weeks until the end of treatment; 0.2 ml of the vehicle three times/week (the control group). Treatment with diethylstilbestrol and polydiethylstilbestrol phosphate produced renal tumors. The antiestrogen nafoxidine administered simultaneously with diethylstilbestrol completely inhibited tumor formation. Hepatic glycuronyltransferase activity towards diethylstilbestrol decreased in hamsters treated chronically with diethylstilbestrol. In hamsters treated with diethylstilbestrol and nafoxidine the activity was not modified. These results indicate that the presence of tumor enhances glycuronyltransferase activity. The treatment with diethylstilbestrol and nafoxidine does not produce statistically significant variations in the activity. This seems to indicate that the antiestrogen counteracts the estrogen-induced decrease in glucuronyltransferase activity.

6716 A COMPARATIVE STUDY OF URINARY OESTROGENS IN THE CANCER OF THE BREAST AND PROSTATE.

(E.) Kaul, P. (Inst. Med. Sci., Banaras Hindu Univ., Varanasi, India), G. C. Prasad, R. C. Gupta and K. N. Udupa. *Indian J Cancer* 11(2):162-167, 1974.

Urinary estrogen excretion was examined in ten normal females and four normal males and compared to 24 hour urinary estrogen excretion in 22 premenopausal and 12 menopausal breast cancer patients, and that in ten prostate cancer patients. Estradiol-17 and estrone excretion was unchanged in premenopausal and post menopausal patients, but estriol was increased over control levels in both groups. Estrogen excretion was measured at an unstated time following bilateral oophorectomy alone or in combination with bilateral adrenalectomy. Both treatments reduced estrogen excretion to values lower than before surgery. The combined oophorectomy and adrenalectomy treatment showed the greatest effect, but traces of estrogens were measurable in the urine. Prostate cancer patients excreted less estrogens than controls, and at an unstated time following surgery, estrogen excretion had not changed. Estrogen formation in organs other than the ovary and adrenal gland is hypothesized due to estrogen secretion observed after the removal of these endocrine glands. An inhibiting factor of the estrogen turnover is suggested to be one of the factors for the causation of prostate cancer in males.

6717 THE EFFECTS OF STEROID HORMONES AND CARCINOGENS ON THE INTERACTION OF MEMBRANES WITH POLYSOMES.

(E.) Rabin, B. R. (Dept. Biochem., U. Coll., London, England), C. A. Blyth, D. Doherty, R. B. Freedman, A. Roobol, G. Sunshine and D. J. Williams. *J Clin Pathol* 27(7):51-59, 1974.

Evidence indicates that free and bound polysomes are probably involved in the biosynthesis of different classes of proteins, and that the ratio of free to bound polysomes is a function of the hormonal status of the organism. Steroid hormones (or equivalent materials extracted from polysomes or membranes) are required for the occurrence of membrane-polysome interactions. It is postulated: 1) that in both sexes estradiol and testosterone are required to be simultaneously present to activate the membranes of polysome binding; 2) that polysomes and/or membranes of the female already contain testosterone, or an equivalent material, and those of the male a substance with properties equivalent to estradiol. There are specific "tight sites" (sites at which the membrane is bound very tightly) for estradiol on the male and for testosterone on the female smooth membranes. These sites are destroyed irreversibly *in vivo* and *in vitro* by aflatoxin B_1, which causes the displacement of polysomes from rough membranes. The estradiol and testosterone, or equivalent substances, are already present in rough reticulum. A number of other carcinogenic chemicals produce the same effects as aflatoxin B_1. Some of these compounds cause degranulation (degranulated rough membranes will rebind polysomes from the same, but not the opposite sex). The presence of NADPH is an absolute requirement. The mechanism of carcinogen activation and subsequent degranulation may involve the cytochrome P450-flavoprotein enzyme present in microsomal membranes. The complete inhibition of the overall degranulation by low concentrations of estradiol suggest that it is the steroid-specific site which is responsible for maintaining membrane-polysome complex integrity; this is attacked by the activated carcinogen.

6718 MOLECULAR EFFECTS OF HORMONES IN BREAST CANCER: ESTROGEN RECEPTORS. *(E.)*

McGuire, W. L. (Univ. Texas Med. Sch., San Antonio), G. C. Chamness and R. E. Shepherd. *Life Sci* 14(1): 19-33, 1974.

The role of estrogen in breast tumor regression, especially the biochemistry of its action, was examined in this review. Data were presented from human mammary tumors and from studies in hormone dependent breast carcinoma induced in rats. Estrogens are probably essential but not sufficient for growth of certain mammary tumors, and in pharmacologic doses estrogen can cause the regression of mammary tumors. Topics that were discussed included: localization of estrogens in target tissues; hormone dependent rat mammary carcinomas; nuclear acceptor activity; and estrogen receptors in human breast tumors. The authors presented a concept of the complete endocrine regulatory system in mammary epithelial cells and hormone dependent breast cancer. The system includes various hormones, e.g. estrogen, prolactin, growth hormone, progesterone, testosterone, corticoids, and insulin. The estrogen receptor data suggested that the loss of endocrine control of breast tumor growth was related to the loss of estrogen receptor.

6719 A MODEL SYSTEM FOR STUDIES OF COLON CARCINOGENESIS: TUMOR INDUCTION BY A SINGLE INJECTION OF METHYLAZOXYMETHANOL ACETATE. *(E.)*

Zedeck, M. S. (Natl. Cancer Inst., Bethesda, Md.) and S. S. Steinberg. *J Natl Cancer Inst* 53(5):1419-1421, 1974.

Immature weanling (50-70 g) male Sprague-Dawley rats (CD line) were given methylazoxymethanol (10 mg/kg in a 0.9% NaCl solution) either i.v. (tail vein) or i.p. to induce tumors of the colon. About 50% of all tumor-bearing animals had progressive weight loss or anal bleeding 8-15 months after treatment. At autopsy, colon tumors were 0.25-1.5 cm in diameter, were almost always pedunculated, and frequently caused intussusception. The tumors had properties similar to those found in man; some were benign adenomatous polyps or adenomatous polyps with focal areas of carcinoma; others were polypoid adenocarcinomas. The incidence of tumors in the colon was almost always greater than that in the small intestine. This could be attributed to differences in bacterial flora that have cocarcinogenic activity or to differences in the distribution or metabolic activation of the carcinogen.

6720 CHROMOSOME REARRANGEMENTS IN THE BONE MARROW DURING THE ANAPHASE FOR THE EVALUATION OF THE MUTAGENIC EFFECTS OF POLYMER BUILDING MATERIALS. *(Rus.)*

Bokov, A. N. (Rostov Med. Inst., USSR), S. I. Gus'kova, E. P. Gus'kov and V. I. Mas'ko. *Gig Sanit* (8):17-20, 1974.

Male Wistar rats were used in the study of the mutagenic effects of RB-2 Indene-Coumarone mastic, UKhM-N bitumen mastic, and two brands of epoxy-based polymer concrete. The animals were exposed 24 hr a day for 90-150 days to the air from the test chamber with the ratio of the material surface area to chamber air volume of 0.3-0.4 m^2/m^3; the temperature was kept at 40-50 C. The following agents were identified (mg/m^3 concentrations): epicholorohydrin (0.027±0.02); styrene (0.005±0.0007); phthalic anhydride (0.034±0.006); benzene (0.52±0.0045); unsaturated hydrocarbons (0.002±0.007); bivinyl (0.026±0.003); and xylene (0.04±0.0007). The analysis of chromosome aberrations at the late anaphase-early telophase stage showed that in the animals exposed to the mastic substances the number of chromosome aberrations was nearly twice as high as in the controls; the incidence of fragments rose from 7.57% (in the controls) to 31.3-39.6%; while the number of bridges in the rearrangements was halved.

6721 THE EFFECT OF *N*-NITROSO CARCINOGENS AND TRIAZENES ON PROTEIN SYNTHESIS IN CELL-FREE SYSTEMS. *(E.)*

Hradec, J. (Oncol. Inst., Prague, Czechoslovakia) and G. F. Kolar. *Chem Biol Interact* 8:243-251, 1974.

The effect of a series of carcinogenic nitrosamines, alkylnitrosoureas, and alkaryltriazenes on the polymerization of phenylalanine, the binding of aminoacyl-tRNA to ribosome, the peptide bond synthesis, and the binding of mRNA to ribosome was studied in cell-free systems from Wistar rat livers. The carcinogenic compounds, in concentrations ranging from 1×10^{-7} to 1 mg/ml, were added into the incubation mixtures as suspensions or solutions in polyethylene glycol. Most carcinogens stimulated the formation of aminoacyl-tRNA complex in test systems whereas analogous preparations from *Escherichia coli* did not show this effect. The polymerization of phenylalanine and the binding of aminoacyl-tRNA to ribosome were only slightly and non-specifically inhibited in the presence of the test compounds, but the binding of mRNA to ribosome was stimulated. The carcinogens intervened specifically with one of the early steps in peptide initiation. It was suggested that the *N*-nitroso carcinogens and triazenes may control the expression of genetic message at the translation level, since it is already known that the binding of mRNA to ribosome is an important rate-limiting step in protein synthesis.

6722 NITRITE IN HUMAN SALIVA. ITS POSSIBLE RELATIONSHIP TO NITROSAMINE FORMATION. *(E.)*

Tannenbaum, S. R. (Dep. Nutrition Food Sci., Massachusetts Inst. Technol., Cambridge), A. J. Sinkskey, M. Weisman and W. Bishop. *J Natl Cancer Inst* 53(1): 79-84, 1974.

Nitrite concentrations in the saliva of five individuals were measured with respect to variation caused by eating and time and the influence of biochemical and microbiological variables; nitrite formation in saliva is considered pertinent to the problem of nitrosamine carcinogenesis. Salivary nitrate concentrations were measured three times in 12 days. Nitrite was consistently found at levels of usually 6-10 ppm. The nitrite level did not seem to be strongly influenced by the composition of a meal, and in some individuals a narrow range of nitrite concentration was maintained for a long time. In agreement with earlier work, parotid ductal saliva was found to be free of nitrite, and a major route of nitrite formation was via microbial reduction of nitrate naturally present in saliva. Exploration of various parameters related to the reduction of nitrate to nitrite did not demonstrate a clear correlation between measurements on 20 individuals and their level of salivary nitrite. *Staphylococcus* and *Micrococcus* were found in largest concentrations, followed by *Veillonella, Nocardia, Cornynebacterium* and an organism tentatively identified as *Fusobacterium nucleatum.* The relationships between nitrate consumption and nitrite formation appeared constant. These complex relationships suggest that the free nitrite in saliva represents the "spillover" of that formed in microbial cells.

6723 QUANTITATIVE STUDIES ON THE IRREVERSIBLE LOSS OF TWO ENZYME ACTIVITIES IN THE RAT LIVER AFTER APPLICATION OF DIETHYLNITROSAMINE. *(Ger.)*

Schieferstein, G. (Max-Planck-Inst. Virusforsch., Tubingen, Germany), J. Pirschel, W. Frank and H. Friedrich-Freksa. *Z Krebsforsch* 82(3):191-208, 1974.

In previous studies of rat liver tumors induced by feeding of diethylnitrosamine (DENA) the formation of islands displaying certain enzyme deficiencies has been noted and a variety of qualitative observations reported. In the present paper the number and areas of the islands in the liver parenchyma with deficiency in nucleoside polyphosphatase (NPPase) and glucoside-6-phosphatase (G-6-Pase) has been examined as a function of the dose in daily feeding over specified periods of time and of a single dose after hepatectomy. Furthermore, differences in the development of these deficiencies in male and female rats, resp., and in different strains have been explored. In continued feeding, 54 Sprague-Dawley rats (180-200 g initial weight) divided into nine groups including controls, received 8 mg/kg/day of DENA in weekly periods (from 1-8 weeks). After 11, 13, and 15 weeks resp., two rats from each group were killed and the livers were examined for deficiency in NPPase and G-6-Pase. An average 5 cm^2 section/animal was evaluated. The insular tissue (expressed in % of total liver tissue) the number of islands, and mean insular areas were found to increase exponentially with the DENA total dose; the curves for the NPP and G-6-Pase deficiencies were superimposable, suggesting a common mechanism. These results were confirmed by introduction (after 2/3 hepatectomy in female Sprague-Dawley rats) of a single dose of 4 mg/kg and 16 mg/kg of DENA resp. The coincidence of the deficiencies were determined after 4, 10, and 16 weeks. In the determination of the island numbers and areas as influenced by the strain and sex of the rats, male and female Sprague-Dawley rats and female BD-IX rats were fed 8 mg/kg/day of DENA for four weeks and the animals were tested at regular intervals after 7-28 weeks. In the liver of Sprague-Dawley females the number and areas of islands were larger by a factor of ten in comparison with the other animals. Reasons for this difference are not known.

6724 THE CORRELATION OF CARCINOGENIC EFFECT AND N-NITROSODIETHYLAMINE DOSE. *(Rus.)*

Likhachev, A. Ia. (N. N. Petrov Sci. Res. Inst. Oncol., Leningrad, USSR). *Vopr Onkol* 20(8):60-64, 1974.

Studies were made of the combined transplacental and postnatal tumorigenic effect of N-nitrosodiethylamine (NDEA) in 42 pregnant albino mice injected i.p. with 30 mg/kg of NDEA dissolved in 0.85% aqueous solution of NaCl 24 - 48 hr before parturition. The progeny were divided into two groups: 57 control mice and 54 mice injected s.c. after one hundred days with NDEA solution (50 mg/kg). The total dose was 300 mg/kg (dose schedule unspecified). In group one, 25 of the 40 mice that survived the latent period developed tumors; in group two, 30 of the 37 survivors developed tumors. The majority had pulmonary neoplasms (22 in group one, and 28 in group two), which were mostly papillary, tubular and trabecular adenomas. The incidence of hepatic tumors was four times greater in group two than in group one (18 and 5, resp.). Gastric tumors were found in two mice from group one and 11 from group two, and were mostly papillomas and squamous cell keratinized carcinomas. There were more primary multiple tumors in group two than in group one (18 and 12, resp.), while the animals in group one had a longer life span (619 days against 443). Thus, injection of NDEA in progeny increased the transplacental tumorigenicity. In previous studies, almost the same results were obtained with mice given four times the dose of NDEA pre- and postnatally. This suggests that pre- and postnatal tumorigenicity are not necessarily additive with such massive doses.

6725 SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF CHANGES IN THE URINARY BLADDER IN RATS TREATED WITH N-BUTYL-N-(4-HYDROXYBUTYL)NITROSOAMINE. *(E.)* Arai, M. (Nara Med. Univ., Japan), T. Kani, S. Sugihara, K. Matsumura, Y. Miyata, Y. Shinohara and N. Ito. *Gann* 65(6):529-540, 1974.

The morphological changes of the urinary bladder epithelium of male Wistar rats induced by N-butyl-N-(4-hydroxybutyl)nitrosoamine (BBN) were examined by scanning and transmission electron microscopy. A 0.05% concentration of BBN was given in the drinking water for 20 wk. Ultrastructural changes were classified into three stages of normal, focal hyperplasia, and transitional cell carcinoma. Transmission electron microscopy showed that cells of normal bladder epithelium had an asymmetric unit membrane and fusiform vesicles, tonofilaments, and many polysomes in the cytoplasm. Regions of focal hyperplasia showed multilayers with clear and dark cells. Regions of carcinoma showed much variation, with dark and clear carcinoma cells, invagination of the cytoplasm, bundles of thin filaments, transformation of the nuclei or nucleoli, and disappearance of fusiform vesicles. Scanning electron microscopy showed that the surface membranes of cells of normal bladder epithelium were smooth with a network of fine ridges. In regions of focal hyperplasia two types of surface were seen; in one type, cells were mostly flat but in places hemispherical cells protruded, while in the other type, the surface cells exhibited a cobblestone arrangement and the presence of many microvilli. In areas of papillary carcinoma the surface was formed from various sized hemispherical cells, and the surface membrane had numerous complex microvilli, unlike normal bladder epithelium.

6726 PHILADELPHIA CHROMOSOME AND GROUP C MARKER IN A CASE OF ACUTE LEUKEMIA ASSOCIATED WITH NORAMIDOPYRINE TREATMENT. *(Fr.)* Moraine, C. (Dep. Cytogenet. Lab. Hum. Biol., Tours, France), J. L. Bremond, F. Despert, J. Leroy, M. E. Leroux and M. Maillet. *Nouv Rev Fr Hematol* 14(4):461-470, 1974.

A case of acute undifferentiated leukemia in a 55-yr-old man is described. The patient was first treated for epigastric pain and fever with antibiotics and noramidopyrine. The noramidopyrine treatment was discontinued when a leukopenia of 2500 elements/mm^3 and a neutropenia of 900/mm^3 were found. Anemia and decreased medullar leukoblastosis ensued, later stabilizing at about 25%. A karyotype study revealed proliferation of a clone carrying a Ph1 Philadelphia chromosome which replaced a member of pair #22 and a supplementary group C marker. The Philadelphia chromosome is generally associated with chronic myeloid leukemia, although in this case no signs of chronic myeloid leukemia were found. Usually, chronic myeloid leukemia eventually became acute, accompanied by development of various chromosomal anomalies. However, there is also evidence for exacerbation of acute leukemia induced by a Ph1 clone independently of the myeloproliferative syndrome. The present case provides data which support this hypothesis of independently induced chronic myeloid leukemia and acute undifferentiated leukemia. The initial iatrogenic medullar aplasia may have revealed or created the group C marker chromosome clone from a preexisting Ph1 clone.

6727 ALVEOLAR CELL HYPERPLASIA IN THE LUNGS OF SMOKING DOGS. *(E.)* Frasca, J. M. (VA Hosp., East Orange, N.J.), O. Auerbach, V. R. Parks and J. D. Jamieson. *Exp Mol Pathol* 21(3):300-312, 1974.

Alveolar cell hyperplasia in the lungs of 14 male beagle dogs that had smoked up to 1810 days was studied. These dogs consumed 11 nonfiltered cigarettes per day after being trained to inhale smoke through a tracheostomy tube. Hyperplasia of bronchiolar epithelium occurred in the lung parenchyma of all smoking dogs, and there was a virtual absence of glycogen in the cell cytoplasm. Sheetlike arrangements of squamous cells containing an abundance of tonofilament bundles were found lining alveoli. Type II cells were found adjacent to and sometimes extending over squamous cells, and there was a proliferation of type II cells in alveolar epithelium, almost completely lining some alveoli. Acinar-like structures also lined by many type II cells were sometimes found near the pleura. Microinvasions of cytoplasmic processes into the underlying connective tissue occurred through defects in the basal lamina. An unusual content of rough endoplasmic reticulum occurred in some type II cells; it possessed electron dense banding ∿ 180 Å wide with a repeat at intervals of ∿ 540 Å and was found in narrow cisternae and in bulbous dilatations of the rough endoplasmic reticular cisternae. Fibrogranular material of similar appearance and periodicity was found in the perinuclear space in some cells. These type II cells were implicated as a reserve cell or stem cell in metaplastic alterations.

6728 ARYL HYDROCARBON HYDROXYLASE INDUCTION IN HUMAN LYMPHOCYTE CULTURES BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN. *(E.)* Kouri, R. E. (Microbiol. Assoc., Bethesda, Md.) and H. Ratrie III. *Life Sci* 15(9):1585-1595, 1974.

The induction of aryl hydrocarbon hydroxylase (AHH) by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3-methylcholanthrene (MC) was studied in purified, phytohemagglutinin or pokeweek mitogen-activated lymphocytes from 19 healthy volunteers. Maximal AAH induction of 1.4 to 2.9-fold was obtained at about 30 nM TCDD, a concentration about 40-60 times less than that of MC (1.5 μM) required for maximal induction. Subjects with higher baseline AHH activities showed a greater degree of enzyme induction by TCDD. Because of the degree of day-to-day experimental variability, variations within each assay, and the inability to delineate distinct classes of low, medium, or high inducibility as found in previous studies for MC, it is suggested that the observed variance of expression of AHH induction more closely fits a unimodal, polygenic (≥ 2 genes) pattern rather than the trimodal (single gene) pattern of inheritance proposed by others. The ability of TCDD to induce AHH in man coupled with its presence in high levels in certain areas of the world suggest that it may present a considerable long-term tumorigenic risk because of possible synergism with other carcinogens.

6729 THE EFFECT OF URETHAN ON THE SYNTHESIS OF NUCLEIC ACIDS IN THE CELLS OF REGENERATING RAT LIVER.

(E.) Rocchi, P. (Inst. Cancerol., Univ. Bologna, Italy), S. Grilli, A. M. Ferreri and G. Prodi. *Z Krebsforsch* 82(4):277-283, 1974.

Partially hepatectomized male Wistar rats were used to study the inhibitory effect of ethyl carbamate (urethan, 1 mg/g) on the synthesis of DNA and RNA. For the study of DNA synthesis, the rats were injected i.m. with urethan 19 hr after the operation and were killed 19 to 53 hr after the operation and, at each time period, 30 min after i.p. injection of ^{3}H-thymidine (0.2 μCi/g). Another group was injected with urethan 23 hr after the operation and killed 2 hr later. For RNA synthesis, the rats were injected with urethan 15 min before the operation and killed at 2 to 40 hr after the operation (30 min after i.p. injection of 5^{3}H-uridine [1 μCi/g]). Another group received both urethan and labeled uridine 19 hr after the operation and were killed 5 hr later. Nuclei for incubation experiments were isolated from uridine-treated animals killed 22 hr after the operation. Total nuclear RNA specific activity was compared with that of non-incubated nuclei. DNA synthesis was first inhibited, then abolished, suggesting that the inhibition by urethan is reversible. No inhibition was observed when urethan was administered 23 hr after the operation. When urethan was injected 15 min before the operation, the total, soluble, mitochondrial and microsomal RNA synthesis was inhibited up to 36 hr after the operation. Nuclear RNA synthesis was higher than normal up to 18 hr after the operation and normal at 40 hr after. The specific activity of the nuclear aqueous and nuclear interphase RNA of rats killed at 18 hr after the operation was greater than normal after incubation. Uridine plus urethan had no effect on RNA synthesis. The results also suggest that urethan affects the processing of some fractions of newly synthesized RNA.

6730 SENSITIVITY OF A LUNG CELL IN THE DEVELOPING MOUSE EMBRYO TO TUMOR INDUCTION BY URETHAN.

(E.) Nomura, T. (Osaka Univ. Med. Sch., Japan). *Cancer Res* 34(12):3363-3372, 1974.

The sensitivity of lung cells of young and fetal mice to urethan-induced tumorigenesis in relation to their degree of differentiation was quantitatively investigated. Forty-nine pregnant ICR/Jcl mice received a single injection s.c. of urethan (1.0 mg/g) on days 7, 9, 11, 13, 15, 17, or 19 of gestation. Mothers and offspring were sacrificed 32 weeks after parturition, and the lungs fixed in formaldehyde and examined for tumors. The tumors were serially sectioned and examined by light microscopy. In another experiment three groups of 12 week old mice were given 1.0 mg/g urethan s.c. in 1, 2, and 3 doses, resp., with four days between doses. Four more groups were given s.c. injections of 0.2, 0.5, 1.0, and 1.5 mg/g urethan, resp. All groups were sacrificed 32 weeks after injection. The 1.0 mg/g dose induced lung tumors in the 12 week old mice, which increased linearly in number up to the second dose, then increased at a greater rate after the third dose. The same pattern was observed in the relationship between the number of tumors and the size of the dose, being directly proportional below 1.0 mg/g and increasing more rapidly after 1.5 mg/g. The ratio of the number of tumors per lobe to the weight of the lobe was directly proportional to the dose and frequency of the urethan treatment. In the fetal lung, the ratio of tumors per lung to the weight of the lung on the day of exposure was inversely proportional to the degree of differentiation from day 13 to day 19. Urethan given within 12 hr of delivery resulted in tumor frequency ten times higher than when the drug was given over 36 hr before delivery, but this was due to the effective retention period of the drug in the fetus being ten times longer than in the mother. Histological study showed that as tumors increased in size, the histological pattern changed from alveolar lining type (round or cuboidal cells) through transitional stages to papillary type (slightly basophilic or columnar cells). It is concluded that the sensitivity of lung cells is inversely proportional to the degree of differentiation, tumor frequency is directly proportional to dose and frequency of urethan, and histological patterns are correlated with size.

6731 URETHAN AS A CARCINOGEN FOR THE EUROPEAN HAMSTER.

(E.) Mohr, U. (Hannover Med. Sch., West Germany), G. Reznik and H. Reznik-Schuller. *J Natl Cancer Inst* 53(5):1359-1367, 1974.

Studies were undertaken to determine whether urethan, like diethylnitrosoamine, induces respiratory tract cancer in the European hamster. Captured European hamsters of both sexes were given i.p. injections of 0.20, 0.10 or 0.05 of the mean lethal dose of urethan (2436 mg/kg) once weekly for life. Only 10/60 hamsters developed respiratory tract neoplasms. However, up to 80% of the animals developed s.c., i.p., and subperitoneal fibrosarcomas; this finding is in contrast to those reported for other laboratory animals. In most instances, the fibrosarcomas infiltrated neighboring tissues and organs and penetrated the lymph nodes, salivary glands, and muscles of the larynx. In 18% of the animals, fibrosarcomas originated from the peritoneal covering of the abdominal cavity and infiltrated the liver, spleen, intestines, capsule of the kidneys, and diaphragm. Five out of eight lung tumors and 6/9 liver tumors were diagnosed as metastases of fibrosarcomas. Ultrastructural studies confirmed the fibrosarcomas to be composed of undifferentiated fibrocytic tumor cells. Four animals developed squamous cell papillomas of the forestomach and four others had renal papillary adenomas. Ten percent of the animals developed adrenal pheochromocytomas that consisted of well-differentiated cells containing phenochrome granules; this proved that the tumors could produce hormones. Such tumors are not found in other laboratory animals after urethan treatment. These results confirm the multipotential carcinogenicity of urethan.

6732 HISTOCHEMICAL AND AUTORADIOGRAPHIC STUDIES OF CELL TRANSFORMATION IN THE RAT CERUMEN GLAND INDUCED BY trans-4-DIMETHYLAMINOSTILBENE AND 3,3'-DICHLORO-4,4'-DIAMINO-DIPHENYLETHER. *(Ger.)* Herrmann, I. F. (No affiliation) and A. Schauer. *Arch Otorhinolaryngol (NY)* 207(2):473-474, 1974.

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6737 CARCINOGENIC ACTION OF BENZO(a)PYRENE AND ITS COMBINATION WITH OTHER ORALLY ADMINISTERED HYDROCARBONS. *(Rus.)* Il'nitskii, A. P. (Inst. Exp. Clin. Oncol., Moscow, USSR), V. M. Voronin and L. G. Solenova. *Vopr Onkol* 20(10):71-75, 1974.

6738 EMBRYOTOXIC, TERATOGENIC AND CARCINOGENIC EFFECTS OF N-NITROSOMETHYLUREA IN RATS. *(Rus.)* Aleksandrov, V. A. (N. N. Petrov Res. Inst. Oncol., Leningrad, USSR). *Vopr Onkol* 20(12):76-82, 1974.

6739 PATHOGENIC EFFECTS OF CHEMICAL AGENTS IN THE ENVIRONMENT. *(Rus.)* Rodionov, G. A. (Res. Inst. Hyg. Toxicol. Pestic. Polymers, Plastics, Kiev, USSR). *Arkh Patol* 36(10):80-89, 1974.

6740 AUTORADIOGRAPHIC STUDY OF THE EFFECT OF 1,2-DIMETHYLHYDRAZINE DI HCL ON DNA SYNTHESIS IN MICE. *(E.)* Shimizu, H. (Eppley Inst. Res. Cancer, Univ. Nebraska, Omaha) and B. Toth. *Fed Proc* 33(3):596, 1974.

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6742 TUMOR PROMOTING EFFECT OF EXTRACT OF REGENERATING RAT LIVER IN RATS TREATED WITH 7,12-DIMETHYLBENZ(a)ANTHRACENE (DMBA). *(E.)* Noval, J. J. (Temple Univ. Health Sci. Cent., Philadelphia, Pa.), F. A. Reichle and R. M. Reichle. *Fed Proc* 33(3):462, 1974.

6743 EFFECTS OF 3-METHYLCHOLANTHRENE (3MC) AND PHENOBARBITAL (PB) ON GLUCURONYLTRANSFERASE (GTase) ACTIVITY OF RAT HEPATOMA AND LIVER. *(E.)* Gessner, T. (Grace Cancer Drug Cent., Roswell Park Mem. Inst., Buffalo, N.Y.). *Fed Proc* 33(3):533, 1974.

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6746 GENETIC DIFFERENCES IN AROMATIC HYDROCARBON-INDUCIBLE 5,5-DIPHENYLHYDANTOIN HYDROXYLASE ACTIVITY IN MOUSE LIVER. *(E.)* Robinson, J. R. (Natl. Inst. Child Health Dev., Bethesda, Md.), S. A. Atlas and D. W. Nebert. *Fed Proc* 33(3):546, 1974.

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6748 ELECTRON MICROSCOPIC STUDIES ON DEGENERATIVE CHANGES AND ATYPICAL NODULES IN RAT PANCREAS INDUCED BY 4-HYDROXY-AMINOQUINOLINE-1-OXIDE (4HAQO). *(E.)* Konishi, Y. (Nara Med. Univ., Kashihara, Japan), R. Sugihara, K. Hirao and N. Ito. *Nihon Rynsho Denshi Kenbikyo Gakkai-Shi* 7(3/4):353-354, 1974.

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6751 AFLATOXIN B_1 HEPATOCARCINOGENICITY IN RAINBOW TROUT EMBRYOS. *(E.)* Sinnhuber, R. O. (Dep. Food Sci. Technol., Oregon State Univ., Corvallis) and J. H. Wales. *Fed Proc* 33(3):247, 1974.

6752 AFLATOXIN B_1 ACTIVATION AND AFLATOXICOL TOXICITY IN RAINBOW TROUT (*SALMO GAIRDNERI*). *(E.)* Schoenhard, G. L. (Dep. Food Sci. Technol., Oregon State Univ., Corvallis), D. J. Lee and R. O. Sinnhuber. *Fed Proc* 33(3):247, 1974.

6753 METABOLISM OF ^{14}C-STERCULIC ACID IN THE RAT. *(E.)* Yoss, J. K. (Dep. Food Sci. Technol., Oregon State Univ., Corvallis), J. E. Nixon, N. E. Pawlowski, D. J. Lee and R. O. Sinnhuber. *Fed Proc* 33(3):248, 1974.

6754 *IN VITRO* EFFECT OF A 5-FLUORODEOXYURIDINE ALBUMIN CONJUGATE ON TUMOUR CELLS AND ON PERITONEAL MACROPHAGES. *(E.)* Barbanti-Brodano, G. (Inst. Microbiol., Univ. Bologna, Italy) and L. Fiume. *Experientia* 30(10):1180-1182, 1974.

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6757 AN ELECTRON MICROSCOPIC OBSERVATION OF EXPERIMENTAL EXOPHAGEAL CARCINOMA OF RATS. *(E.)* Ichikawa, T. (Tokyo Women's Med. Coll., Japan), N. Sakakibara and T. Nagayo. *Nihon Rynsho Denshi Kenbikyo Gakkai-Shi* 7(3/4):315-316, 1974.

6758 THE INDUCTION OF ENZYMES IN RAT LIVER MICROSOMAL FRACTIONS AFTER PRETREATMENT WITH AFLATOXIN B_1. *(E.)* Bababunmi, E. A. (Univ. Ibadan, Nigeria), G. O. Emerole, O. Bassir and A. Gershlick. *Fed Proc* 33(3):559, 1974.

6759 BILE ACID CONTENT OF INTESTINAL TISSUE AND FECES DURING AZOXYMETHANE CARCINOGENESIS IN THE RAT. *(E.)* Nigro, N. D. (Wayne State Univ. Sch. Med., Detroit, Mich.), V. M. Sardesai, C. Chomchai, R. L. Campbell and H. Provido. *Fed Proc* 33(3):260, 1974.

6760 ALBUMIN UPTAKE BY MOUSE SARCOMA CELLS IN CULTURE: ENHANCEMENT BY POLYSTYRENE LATEX PARTICLES. *(E.)* Rothman, S. W. (Boston Univ. Sch. Med., Mass.) and H. J. P. Ryser. *Fed Proc* 33(3):556, 1974.

6761 POTENTIALLY CARCINOGENIC CYCLOPENTA[*a*]-PHENANTHRENES. PART IX. CHARACTERISATION OF A 5,10-EPOXYBENZOCYCLODECENE AS A MAJOR URINARY METABOLITE OF THE CARCINOGEN 15,16-DIHYDRO-11-METHYLCYCLOPENTA[*a*]PHENANTHREN-17-ONE. *(E.)* Coombs, M. M. (Imp. Cancer Res. Fund Lab., London, England) and F. E. H. Crawley. *J Chem Soc [Perkin I]* 20:2330-2335, 1974.

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6763 HEPATIC AND EXTRAHEPATIC INDUCTION OF SELECTED MICROSOMAL ENZYMES BY THREE ANTIOXIDANTS. *(E.)* Cremin, F. (Massachusetts Inst. Technol., Cambridge) and S. A. Miller. *Fed Proc* 33(3):228, 1974.

6764 INTERACTIVE MUTAGENIC EFFECTS OF DIMETHYLAMINE, METHYLUREA, ETHYLUREA AND SODIUM NITRITE. *(E.)* Couch, D. B. (Dep. Pharmacol., Virginia Commw. Univ., Richmond) and M. A. Friedman. *Fed Proc* 33(3):234, 1974.

See also:

* (Rev): 6601, 6606, 6607, 6608, 6609, 6610, 6611, 6612, 6613, 6614, 6615, 6653, 6658, 6661, 6662, 6663, 6666, 6667, 6668, 6669, 6670, 6671, 6673, 6674, 6675, 6676, 6684
* (Phys): 6780
* (Viral): 6819
* (Immun): 6891, 6893
* (Path): 6986, 7043, 7073
* (Epid-Biom): 7077, 7080, 7091

6765 INFLUENCE OF THE THYMUS AND OF ALS ON RADIATION LEUKEMOGENESIS. *(E.)* Van Bekkum, D. W. (Radiobiol. Inst. TNO, Rijswijk, Netherlands) and H. Balner. *Bibl Haematol* (39):677-688, 1973.

The susceptibility of mouse to the direct induction of leukemia by virus and the indirect leukemia induced by whole body irradiation was investigated to determine whether the latter is due to genetically determined factors in the thymus or to factors located in other tissues. There was a high incidence of late lymphomas which occurred independently of the thymus in thymectomized (CBA X C57BL)F_1 hybrid mice receiving four weekly irradiations of 180 r each. These tumors were not observed in non-irradiated mice of the same hybrid stock. The occurrence of reticular cell tumors was higher in the thymectomized F_1 group than in the groups receiving thymic implants, which suggests that the development of this malignancy was facilitated by a decreased immune reactivity of the recipient. Prolonged treatment with rabbit ALG reduced the incidence of early lymphomas in another study with F_1 hybrid mice following exposure and in the present experiments in RFM mice following a single radiation exposure. This reduction was more pronounced in the F_1 hybrid mice than in the RFM mice, which might be due to the unexpected enhancing effect of NRG in the latter. If the enhancing factor in early lymphoma induction in RFM was also present in the ALG, the inhibitory factor in the same serum might have been counteractive to a certain extent. A speculation which involves the same target cell for the two factors is that ALG inactivates a certain proportion of precursor or even stem cells of the hemopoietic system, while foreign serum in general might stimulate the proliferation of such stem cells. The latter phenomenon has been described following administration of a variety of foreign sera and other antigens. The existence of such a mechanism would seem to support the hypothesis that the hemopoietic stem cell is the direct target cell for leukemic viruses, as has been proposed by Brommer and Bentvelzen for the Rauscher leukemia virus.

6766 RADIATION-INDUCED CHANGES IN THE DIVISION KINETICS OF THE LIEBERKÜHN CRYPT CELLS WITH SPECIAL RESPECT TO THEIR MIGRATION RATE INTO THE VILLI. *(E.)* Unger, E. (Frederick Joliot-Curie Natl. Res. Inst. Radiobiol. Radiohyg., Budapest, Hungary) and L. Varga. *Strahlentherapie* 147(4):426-431, 1974.

The labeling and mitotic indices of Lieberkühn crypt cells and their migration rate towards the villi were studied by ^{3}H-thymidine autoradiography in the small intestine of 250 R irradiated CFLP mice. Irradiation induced changes in the number of M- and S-phase cells, although the alterations were slighter than those reported in literature. These changes were reflected also in the migration rate of the cells. A slight decrease in migration rate, as compared to controls, occurred at 12 hr; this is assumed to be a consequence of postirradiation mitotic inhibition. The migration rate appreciably exceeded the control values at 72 hr, but the rate again dropped after this time. These changes in migration rate might be correlated with the parallel changes of the mitotic index, which had occurred 10-48 hr. earlier. Since in the experiments reported the DNA precursor had been injected to all the experimental groups at the same hour, between 8 to 9 a.m., corresponding to the various time points counted from the hour of the injection, the processing of the animals was necessarily performed at various hours of the day. Still, no appreciable differences showed either in the labeling or mitotic indices and no circadian alterations in the parameters studied could be demonstrated.

6767 EFFECT OF IRRADIATION ON THE THYROID CELL PROLIFERATION IN RATS. *(E.)* Christov, K. (Inst. Pathol., Univ. Freiburg, West Germany). *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna. April 4-6, 1973* 1:40-45, 1974.

The extent to which changes in the thyroid gland induced by irradiation and treatment of the animals with goitrogens are associated with changes in the number of dividing cells and DNA synthesis was investigated. The thyroid gland of Wistar rats was irradiated with a total dose of 300 rads. Irradiation caused no significant change in the thyroid wt, labeling index and total ^{3}H-thymidine incorporation. Treatment of the animals with 0.1% solution of methylthiouracil as drinking fluid induced an increase in the weight, labeling index, and the total ^{3}H-thymidine incorporation. Thus, the curve for the thyroid weight consists of a lag phase for two days, an exponential phase for about 6-8 days and a plateau phase until the end of the experimental period (28th day). Changes of the labeling index and ^{3}H-thymidine incorporation also indicate a lag phase of two days, an exponential phase with a peak between the sixth and the eighth day and a sharp decrease between the 10th and the 16th day. The values of labeling and DNA synthesis between the 16th and 28th day, were higher than those of the controls, but remained constant. Doses of 300 rads x-rays decreased the growth response of the thyroid gland to MTU stimulus and increased, at the same time, the number of dividing cells and ^{3}H-thymidine incorporation. In terms of the mode of action of x-rays it is suggested that radiation stimulates the cells to enter the cell cycle, but does not allow them to pass into mitosis. Thus, the number of cells with higher-than-normal values of DNA (cells in S and G_2 phases) increases, but the number of mitoses decreases.

6768 MALIGNANCY OF THE UTERINE CORPUS FOLLOWING IRRADIATION THERAPY FOR SQUAMOUS CELL CARCINOMA OF THE CERVIX. *(E.)* Fehr, P. E. (Dep. Obstet. Gynecol., Univ. Minnesota Hosp., Minneapolis) and K. A. Prem. *Am J Obstet Gynecol* 119(5):685-692, 1974.

Records of 2294 patients treated at the University of Minnesota Hospitals for squamous cell carcinoma of the cervix between 1939 and 1966 were reviewed for evidence of malignancy of the uterine corpus. There were 12 patients with proved malignancies of the uterine corpus, an incidence of 0.52%. There were six other patients who received radiation therapy elsewhere for squamous cell carconoma of the cervix who were referred for treatment after discovery of a corporeal malignancy. None of these 18 patients had received estrogen therapy at any time after primary radiation therapy. The interval between radiation therapy for squamous cell carcinoma of the cervix and discovery of the corporeal malignancy ranged from 5-18 yr, with an average of 10.9 yr. Only four patients had vaginal bleeding, and one had a blood-tinged discharge. The absence of bleeding in eight patients is attributable to stenosis of the cervical canal that resulted from the massive radium radiation. Because bleeding was occult in such cases, the disease was more advanced before it produced significant symptoms that impelled the patient to seek gynecologic consultation. This delay resulted in a much poorer prognosis. In a further analysis, 1022 of the 2294 patients died within 5 yr of radiation therapy. Of those who died, 29 (2.8%) had a second primary malignancy which was the cause of death in 20. All but one of these second primary tumors were extrapelvic. Among the 1272 patients who survived more than five yr, there were 70 (5.5%) second primary malignancies of which 30 were in the pelvis. In 64 of these patients, the second primary malignancy was the cause of death. A comparison of this study with two other shows agreement on the importance of vaginal bleeding as a prognostic index. Though the over-all results are uniformly bad, the longest survivors were those who presented with vaginal bleeding or abnormal cytology.

6769 A DIFFERENCE BETWEEN B10.D2/N AND B10.D2/O STRAINS OF MICE IN RATES OF IRRADIATION-INDUCED LEUKEMOGENESIS.

(E.) Kallis, N. (Jackson Lab., Bar Harbor, Me.), H. Meier, S. H. Langley and H. S. Shin. *Cancer Res* 34(12):3210-3214, 1974.

The rates of irradiation-induced leukemogenesis were investigated with mice of the congenic strains B10.D2/n and B10.D2/o. Groups of 25 females of each strain received a total of 1000 R of x-rays in divided doses. In three of four trials, leukemogenesis rates were significantly higher in B10.D2/n females than in B10.D2/o females. B19.D2/o mice lacked complement component C5 while B10.D2/n mice were normal for complement. Spleens of young females of both strains were negative for type C RNA virus *gs*-antigen. In contrast, most spleens of leukemic mice were *gs*-antigen positive. All virus isolates were B-trip; i.e., they grew on BALB/c embryo fibroblast cultures but not on SWR embryo cells. The irradiation protocol appears to be significant for emphasizing the divergence between the two strains. The most highly significant difference in rates occurred at a total dose of 600 R; the smallest occurred at total dose of 1000 R. Irradiation-induced leukemogenesis supports the concept of immune surveillance since the presumed surveillants (thymus derived cells) are sensitive to irradiation. The seeming paradox in these results is the higher rate of leukemogenesis in the B10.D2/n strain, which is normal for complement. The resolution of the paradox may lie in the suggestion of a weak immune response which actually may stimulate tumor growth. Complement levels are not depressed in mice exposed to a single dose of 500 R s-irradiation, and it is possible that a normal complement constitution may be an adjunct for a weakened and discommoded histocompatibility reaction. The data of the fourth experiment support this conclusion: the lowest total irradiation dose (600 R) produced the largest divergence between the two strains of mice. Involvement of C5 is speculative.

6770 MYELOGENOUS LEUKEMIA AND RELATED MYELOPROLIFERATIVE DISORDERS IN BEAGLES CONTINUOUSLY EXPOSED TO ^{60}CO γ-RADIATION.

(E.) Fritz, T. E. (Div. Biol. Med. Res., Argonne Natl. Lab., Ill.), W. P. Norris and D. V. Tolle. *Bibl Haematol* (39):170-188, 1973.

Myeloproliferative lesions resembling myelogenous leukemia are described in an experiment on the response of young adult beagles of both sexes to continuous whole-body irradiation by ^{60}Co γ-rays at rates varying from 5-300 r/22 hr exposure day. Thus far, dogs at the lowest exposure have been irradiated for up to 1200 days. All deaths resulted from damage to the hematopoietic system manifested as three distinct syndromes: septicemia, anemia and myeloproliferative disease usually in the form of myeloid leukemia. The causes of death were related to the exposure rate and total accumulated dose which are reflected in the time of survival. At exposure rates of 35 r/day and above, all deaths resulted from septicemia, but septicemia does not occur at 10 r/day or less. At 17 r/day all three terminal syndromes were seen. At 10 and 5 r/day, only anemia and myeloproliferative disease were seen, with myeloproliferative disease occurring at significantly longer times of exposure. When exposed till death at 17 r/day, two of 13 decedents had myeloproliferative disease after a mean 1038 days of irradiation, while at 10 r/day six of 13 decedents had myeloproliferative disease after a mean of 719 days of irradiation. Only six of 24 dogs being irradiated at 5 r/day died; four had myeloproliferative disease at a mean of 1,063 days. These results demonstrate that the response of bone marrow varies with both dose rate and total dose. The data show that hematopoiesis in beagles, judged by peripheral blood values, tends to equilibrate with continuous irradiation, particularly at rates of 5-10 r/day. Myeloid leukemia and related myeloproliferative disease were observed only in those dogs whose blood values indicated such an attempt at equilibration.

6771 DOSE-RESPONSE RELATIONSHIP OF LYMPHOCYTE CHROMOSOME ABERRATIONS IN LOCALLY IRRADIATED PERSONS.

(E.) Matsubara, S. (Sch. Med., Tokyo Med. Dent. Univ., Japan), M. S. Sasaki and T. Adachi. *J Radiat Res (Tokyo)* 15(4):189-196, 1974.

The dose response relationship of chromosome aberrations in the peripheral blood lymphocytes was investigated in blood samples obtained from 35 patients who were exposed to a single dose of ^{60}Co γ-rays for their malignant diseases. In all cases, 24 hr after irradiation, a significant number of chromosome aberrations were observed. They included tricentrics, dicentrics, rings, acentric fragments and abnormal monocentrics. On the simple per-cell basis, the frequencies of dicentrics and rings varied from 0.0004 to 0.052 per cell. There was no clear cut dose response relationship between radiation dose and the frequencies of chromosome aberrations when they were expressed on the simple per cell basis. Body weight, irradiatied volume and radiation induced inactivation of lymphocytes were other important factors intimately related to the frequencies of chromosome aberrations. Aberration yield expressed on the simple per cell basis was roughly proportional to the irradiated volume and inversely proportional to the body weight. The intercomparison of the frequency of chromosome aberrations in these locally irradiated persons with that obtained by the *in vitro* experiments suggest the D_{37} value of about 350 rads for the survival of lymphocytes as measured 24 hr after irradiation. The results indicate that the initial ratio of irradiated to unirradiated lymphocytes in the extra-vascular lymphocyte pool and the radiation induced interphase death of the lymphocytes are the most important factors determining the chromosome aberration yield in peripheral blood lymphocytes.

6772 MORPHOLOGICAL EVALUATION OF ^{226}RA INDUCED OSTEOGENIC SARCOMA IN MICE. *(E.)* Holusa, R. (Inst. Hyg. Epidemiol., Prague, Czechoslovakia), V. Kofranek and Z. Onyskowova. *Neoplasma* 21(6):697-709, 1974.

Two groups of random bred SPF female mice of H strain injected intraperitoneally with two doses of ^{226}Ra activity (8.2 μCi/kg and 20 μCi/kg) were histologically examined and bone tumors as a specific effect of radionuclide incorporation were evaluated morphologically. In the group of 122 mice injected with lower radioactivity 20 bone tumors (19 osteosarcomas and one ossifying fibroma of mandibula) and in the group of 94 mice injected with the higher radioactivity 17 osteosarcomas were determined. In the control group of 72 mice one spontaneous osteosarcoma was found. In the incidence there were no differences with regard to the injected radioactivity. Most of the induced osteosarcomas were localized in the axial skeleton (59.4%). From the jaws arose six tumors. A detailed histological examination of all osteosarcomas led to the conclusion that besides undifferentiated proliferating cellular components of the tumor, the more mature parts were formed by bone and collagenous connective tissues and to a lesser extent also by cartilage. The formation of osteoid or of more or less mature bone traceculae was determined as a characteristic diagnostic symptom. Although the histological structure and extent of osteoplasia differed from one tumor to the next, it was not possible to divide the radiation-induced sarcomas into subclasses based on different characteristics, and all were viewed as a single oncological class. Further, with the exception of the mandibular tumor, histological pattern could not be correlated with anatomic location.

6773 ULTRASTRUCTURE OF ^{90}Sr INDUCED OSTEOSARCOMAS AND EARLY PHASES OF THEIR DEVELOPMENT. *(E.)* Nilsson, A. (Div. Radiat. Biol., Res. Inst. Natl. Def., Sundbyberg, Sweden), P. Sundelin and I. Sjoden. *Acta Radiol* 13(2):107-128, 1974.

Ultrastructural characteristics of ^{90}Sr induced malignancy in osseous and reticular tissues have been investigated in CBA- mice. A clear cellular pleomorphism was identified (stage III, solitary or multiple small buds of tightly-packed morphologically malignant cells inside non-neoplastic fibrous tissue). During tumor progression the characteristics of the cells differed more and more with respect to every parameter with increasing stage development. At stage VI (tumors infiltrating and breaking through compact bone) and VII (overt, paraosteal tumors) an increasing population of anaplastic cells was noted. The earliest ultrastructural changes were noted in proliferating cells in stage I (hypoplastic bone marrow with swelling and increasing number of reticular cells) in the form of folding of the nuclear membrane. In successive stages reticular cells gradually adopted ultrastructural traits which characterize cancer cells: hypertrophy of the nucleus and the nucleolus, irregularities in the chromatin distribution, increase in ribosomes and increased numbers of mitochondria. The peak number of mitochondria occurred in stage IV (fibroblastic osteosarcomas within the bone marrow), slightly preceding the peak number of ribosomes in stage V (similar characteristics to stage IV), which coincided with maximum cytoplasmic ultraviolet extinction. The peak number of lysosomes also occurred around this time, all of which reflect the maximum proliferating activity at stages IV and V. Virus-like particles were found in three separate tumors, all at stage VII. It is suggested that the virus is not the initiator of carcinogenesis, but rather is simply carried along with the tumor, for the antiviral defense inside a tumor is severely impaired and this creates a safe residence for many types of virus.

6774 LUNG CANCER AND URANIUM MINERS. *(E.)* Delpla, M. (Electricity France, Paris) and S. Vignes. *Radiat Res* 59(1):223, 1974.

6775 ILLUMINATION AND X-RAY EFFECTS ON TUMORIGENESIS IN RODENTS. *(E.)* Hellman, K. B. (Bur. Radiol. Health, Rockville, Md.) and C. D. Lytle. *Radiat Res* 59(1):20, 1974.

6776 THE EFFECT OF HETEROCHROMATIC ULTRAVIOLET MICROBEAM IRRADIATION UPON DNA SYNTHESIS OF THOSE CELLS EXHIBITING NORMAL AND ABNORMAL DIVISION. *(E.)* Algunes, C. (Dep. Radiobiol., Univ. Istanbul, Turkey). *Istanbul Univ Fen Fak Mecm Seri B* 39(1/2):73-93, 1974.

6777 EXTRAPULMONARY TUMORS IN RATS INDUCED BY INTRATRACHEAL ADMINISTRATION OF ^{241}AMERICIUM. *(Rus.)* Filippova, L. G. (No affiliation) and A. P. Nifatov. *Radiobiologiia* 14(5):793-796, 1974.

6778 TRAUMATIC TUMORS OF THE NERVOUS SYSTEM AND ITS SHEATHS WITH EMPHASIS ON ONE CASE OF A ROOT NEURINOMA NEAR A BULLET. *(Ger.)* Holzer, P. (Neurosurg. Clin., Univ. Graz, Austria). *Zentralbl Neurochir* 35(1):55-59, 1974.

6779 CYTOGENETIC ANALYSIS OF THE ANTIMUTAGENIC EFFECT OF α-TOCOPHEROL ON SPONTANEOUS AND RADIATION-INDUCED CHROMOSOME MUTATIONS. *(Rus.)* Alekperov, U. K. (V. L. Komarov Inst. Botany, Baku, USSR) and D. D. Akhundova. *Genetika* 10(7):12-18, 1974.

6780 EXPERIMENTAL METHODS -- CELL AND TISSUE CULTURE: EFFECTS OF ASBESTOS PARTICLES ON MACROPHAGES, MESOTHELIAL CELLS AND FIBROBLASTS. *(E.)* Allison, A. C. (Clin. Res. Cent., Harrow, England). In: *Biol Eff Asbestos, Proc Work Conf, 1972* edited by P. Bogovski, V. Timbrell, J. C. Gilson, J. C. Wagner. Lyon, France, International Agency for Research on Cancer, 1973, pp. 89-93. (9 references)

6781 HEPATIC CHOLANGIOCARCINOMA INDUCED IN SYRIAN HAMSTERS BY THOROTRAST. *(Jpn.)* Okamoto, T. (Kanagawa Prefecture Adult Dis. Cent., Japan). *Nippon Acta Radiol* 34(4):254-265, 1974.

6782 RADIATION INDUCED CANCER OF THE THORACIC ESOPHAGUS. *(Jpn.)* Asakawa, H. (Radiol. Dep., Miyagi-ken Seijinbyo Cent., Miyagi-ken, Japan), H. Otawa and J. Watarai. *Gan No Rinsho* 20(5):413-416, 1974.

See also:

* (Rev): 6609, 6660, 6664, 6665, 6666, 6667, 6674
* (Viral): 6826
* (Path): 6983

6783 USE OF LEUKOCYTE CULTURES TO ISOLATE VIRUSES IN HUMAN LEUKEMIA. *(Rus.)*

Shubladze, A. K. (D. I. Ivanovskii Inst. Virol., Moscow, USSR), I. F. Barinskii, A. F. Bocharov, E. P. Ugriumov, F. P. Filatov, G. A. Delimbetova and V. M. Zhdanov. *Vopr Virusol* (6):719-724, 1973.

WBC from eight patients with acute hemocytoblastosis and from 85 patients with various forms of chronic leukemia were cultured in medium 199 containing autologous or homologous serum together with donor WBC which had been stimulated by phytohemagglutinin. During the fifth to the 15th passages, five strains of oncornavirus with type A and C morphology were observed in cells and culture fluid which contained WBC from eight patients with acute leukemia, while no such particles were found in WBC cultures from 20 nonleukemic donors. Reverse transcriptase activity was detected in two virus strains cultivated with exogenous poly- and oligonucleotides, and this was confirmed by ratios of dA/gA of 1.9 to 8.3. In studies with endogenous oligo- and polynucleotides, reverse transcriptase activity was associated with 70S RNA. Mitotic activity in 72-hr cultures of normal donor WBC was significantly increased when they were infected with type A and C oncornavirus. Of 85 attempts to culture WBC from patients with various forms of chronic leukemia, only four were successful. In all four cases the WBC were from patients with chronic myeloid leukemia; all four of these cultures were monolayers which could be passaged only a few times. WBC from two patients survived for up to seven months. Type A and C particles with diameters of 870-1300 Å were observed in ultrathin sections of these cells. Oncornaviruses isolated from these cells were inoculated into primary trypsinized human fibroblasts and passaged 20-30 times in human skin and muscle cultures grown in Eagle's medium containing 10% bovine serum. Labeling with ^{3}H-uridine demonstrated that virus particles had a buoyant density of 1.15-1.17 g/cm^3. Their synthesis was inhibited by actinomycin D, and the RNA which they contained sedimented in a 10-30% sucrose gradient at 70S.

6784 COEVOLUTION OF RNA TUMOR VIRUS GENOME AND VERTEBRATE HOST GENES. *(E.)*

Gilden, R. V. (Flow Lab., Inc., Rockville, Md.) and S. Oroszlan. *Collect Pap Annu Symp Fundam Cancer Res* 25:460-476, 1974.

To determine whether evolutionary relationships among species can be established on the basis of homologies between C-type viruses orignating in different species, comparisons were made of physical and chemical properties of the major group-specific (gs) protein isolated from mouse, hamster, rat, cat, chicken, Russel's viper, RD-114, Gibbon ape, and Woolly monkey viruses. Points of similarity of preparations from the different sources consisted of a narrow range of molecular weights (25,000-35,000), comparable results on isoelectric focusing, and occurrence in common of antigenic gs-3 determinant and of proline as the amino terminal of the gs protein. Points of dissimilarity include distinct isoelectric points and species-specific gs-1 determinants of gs proteins from the different viruses. Key features of the results obtained from amino acid sequence studies on p30 proteins were the obvious over-all homology, a hypervariable region (residues 4-10), and an invariant region (residues 11-25). The Gibbon ape virus sequence differed from the others in requiring 6-7 insertions before reaching the completely homologous region of the sequence. It appeared significant that three of the first four positions of feline leukemia virus and mouse leukemia virus were identical while two of ten were identical in avian and murine viruses. The evidence obtained for the RD-114 virus indicated that it was of feline, not human, origin, but that it was distinct from other viruses tested on the basis of antigenicity of polymerase, partial inhibition of gs-3 reaction between goat anti-feline leukemia virus and murine leukemia virus on radioimmunoassay, and a gap at the 5' position in the amino acid sequence compared to subprimate viruses. The results indicated divergence of three major groups of mammalian type C viruses, namely, mouse-rat-hamster-cat, RD 114, and ape-monkey. While the assumption of a single type-C virus per species was not valid, the virus proteins themselves remain as a homologous class and therefore subject to the types of analysis described.

6785 MOLECULAR STUDIES OF CELLS DERIVED FROM HUMAN SOLID TUMORS. *(E.)*

Allen, P. T. (Dep. Virol., Univ. Texas M. D. Anderson Hosp., Houston), W. A. Newton, J. L. East, K. Maruyama, J. M. Bowen, J. Georgiades, E. S. Priori and L. Dmochowski. *Collect Pap Annu Symp Fundam Cancer Res* 25:531-546, 1974.

Based on the suggestions of Gallo and of Spiegelman that the assay for virus-associated DNA polymerase of RNA tumor viruses may be a more sensitive method for detecting the viruses than electron microscopy, supernatant fluids from cell cultures derived from human tumors were examined for the presence of the enzyme. The culture fluids were first concentrated by single-phase polyethylene glycol precipitation. The concentrates were then centrifuged onto sucrose cushions designed to retain particulate components having the density range of RNA tumor viruses. It was found that inoculation of cells derived from certain types of human tumors (fibrosarcoma desmoides, giant cell tumor) with bone marrow aspirates from patients with acute lymphocytic leukemia resulted in transformation of the target cells. These transformed cells, as well as the uninoculated parent cells, release particles with endogenously instructed reverse transcriptase activity. This activity of the transformed cells was, however, significantly higher than that of the parent cell lines and was associated with particles having many properties characteristic of RNA tumor viruses. An explanation for the transformation following inoculation with leukemic bone marrow cells and for the presence of particles with reverse transcriptase activity in the transformed cells was suggested to be the activation of a covert sarcoma virus genome in the susceptible cells at the time of transformation. The role of the particles in transformation has yet to be ascertained. The results of the study added further evidence for the presence of a viral agent(s) in human tumors of the types studied and opened up the possibility of applying molecular probes to a wider variety of human tumors.

6786 COMPARISON AND EVOLUTION OF RNA TUMOR VIRUS COMPONENTS. *(E.)* Gilden, R. (No affiliation), S. Oroszlan and M. Hatanaka. In: *Viruses, Evolution and Cancer: Basic Considerations* edited by E. Kurstak and K. Maramorosch (New York, Academic Press, 1974), pp. 235-257.

Immunochemical and molecular hybridization techniques were applied in studies on the relationships and characteristics of RNA tumor viruses. Uncertainty exists concerning the evolution of RNA tumor viruses in mammals. Known viruses of mice and rats fulfill the criteria of inherited viruses, while the hamster viruses have not yet been adequately tested. Reported results indicate that viruses isolated from a gibbon ape (GaLV) and a woolly monkey (WoLV) may fail to meet criteria of being represented in cellular DNA: this is of significance in deriving evolutionary relationships in host species and in seeking knowledge on human type C viral genetic information. The high degree of relationship among primate viruses is supported by hybridization studies, results of antigenic assays for the virion reverse transcriptase, and alternate methods of assay. In contrast to guinea pig antisera, which are highly species-specific, certain goat anti-p30 sera are highly crossreactive in immunoassays. There is obvious homology among all the p30's examined which gives support to a common ancestry hypothesis. Current evidence that WoLV and GaLV are not inherited viruses in primates helps explain present inability to find homologous nucleic acid sequences in human cellular DNA. (64 references)

6787 INFIDELITY OF DNA SYNTHESIS: A GENERAL PROPERTY OF RNA TUMOR VIRUSES. *(E.)* Sirover, M. A. (Inst. Cancer Res., Philadelphia, Pa.) and L. A. Loeb. *Biochem Biophys Res Commun* 61(2): 410-414, 1974.

The frequency with which a non-complementary base-paired nucleotide is incorporated by the DNA polymerase of Rauscher leukemia virus was determined using synthetic polynucleotides as templates. With poly(rA).oligo(dT) as template, the DNA polymerase incorporated one molecule of non-complementary base-paired deoxyribonucleotide for every 525 molecules of complementary nucleotide polymerized. When a purine non-complementary nucleotide was used, the error rate using poly(rA).oligo(dT) as a template was reduced to less than 1/3600. With poly(rC).oligo(dG) as template, the enzyme incorporated one molecule of non-complementary base-paired deoxyribonucleotide for every 307 molecules of complementary nucleotide polymerized. These error rates are of the same magnitude as those previously reported for avian myeloblastosis virus DNA polymerase but are significantly higher than those reported for *E. coli* DNA polymerase. It is suggested that a high level of infidelity may be a common characteristic of RNA tumor viruses. It is further suggested that this capability to incorporate errors into DNA may be relevant to the molecular mechanism for tumor induction by the oncogenic RNA viruses.

6788 TEMPLATES AND PRIMERS FOR THE VIRION DNA POLYMERASE OF RNA TUMOR VIRUSES. *(E.)* Baltimore, D. (Dep. Biol., Massachusetts Inst. Technol., Cambridge), I. M. Verma, D. F. Smoler, I. Molineux, N. N. Meuth, H. Y. Fan, and G. F. Temple. *Collect Pap Annu Fundam Cancer Res* [25:221-228, 1974]

An analysis was made of the role of the primer for virion DNA polymerase of RNA tumor viruses and of the ability of different primer template combinations to direct DNA synthesis. It appears that there is a polyribonucleotide primer in the 60-70S RNA complex. The primer is probably not any of the major 37S subunits of the 60-70S RNA, since these appear to end in uridylate at their 3'-ends. The primer does not end with poly(A) because it is hydrolyzed by ribonuclease in high salt. The best template yet found for the virion DNA polymerase is the 10S RNA from reticulocytes of numerous animal species. The 10S RNA contains the globin messenger RNA, and it is assumed that the messenger RNA is the active fraction. The significance is apparent of the dual primer and template requirements for virion DNA polymerase. For synthesis to occur, both polynucleotides are needed, except in cases such as poly(dA-dT), in which the self-complementarity of the molecule permits it to be both primer and template. Oligonucleotides or polynucleotides that contain deoxyribonucleotides or ribonucleotides can act as primers. Templates can similarly contain deoxyribose or ribose.

6789 A NEW APPROACH TO THE ISOLATION OF RNA-DNA HYBRIDS AND ITS APPLICATION TO THE QUANTITATIVE DETERMINATION OF LABELED TUMOR VIRUS RNA. *(E.)* Coffin, J. M. (Inst. Mol. Biol. Univ. Zurich, Switzerland), J. T. Parsons, L. Rymo, R. K. Haroz and C. Weissmann. *J Mol Biol* 86(1):373-396, 1974.

A procedure has been developed by which the hybrid formed between a labeled RNA and complementary DNA can be selectively separated from all other single and double-stranded nucleic acids using Vero cells (African green monkey cell line), the Schmidt-Ruppin strain of Rous Sarcoma virus (subgroup D), *Escherichia coli* and Q13 avian myeloblastosis virus (BAI strain A). Vero cells were cultured and labeled with uridine. *E. coli* was infected with bacteriophage T4 (9 plaque-forming U/cell), uridine was labeled and RNA was extracted. Cyanogen bromide (15 g) was added to 50 ml of Sephadex and allowed to react for 15 min. The Sephadex was suspended in 0.2 M-sodium 2-(N-morpholino) ethane sulfonate buffer containing 15 mg of Poly I. The polyinosinic acid [Poly(I)] was found to be covalently linked to Sephadex. Labeled homopolymers, [^{3}H]poly(C), [^{3}H]poly(U) and [^{14}C]poly(G) were synthesized using polynucleotide phosphorylase from *E. coli* and the appropriate labeled ribonucleoside diphosphate. The poly(I) retains the specific RNA-DNA hybirds by virtue of their poly(C) extension. The column was washed with RNAase to degrade non-hybridized RNA; the RNA retained on the column was eluted with formamide and its radioactivity was determined. The background hybridization was reduced to 0.003 to 0.008% by addition of oligo(C) to the hybridization mixture and by carrying out the adsorption to the poly(I)-Sephadex column in the presence of poly(U). The efficiency of hybridization was about 50%. The radioactive Rous Sarcoma virus-specific RNA content was determined in infected and uninfected cells after two hr labelling with (^{3}H)uridine. In infected cells the content was 0.6 to 0.9% while in uninfected cells the content was only 0.05%. The content for monkey cell RNA was found to be 0.009%. This method can be used for the detection of hybrids between labeled RNA and complementary DNAs too short to be adsorbed to nitrocellulose filters to allow quantitation by conventional methods. The author suggests that with the omission of the RNAase step, this procedure can be used for the isolation of any RNA for which a complementary DNA is available, as well as for its precursor.

6790 SARCOMA-VIRUS-RELATED RNA SEQUENCES IN NORMAL RAT CELLS.

(E.) Tsuchida, N. (Flow Lab., Rockville, Md.), R. V. Gilden and M. Hatanaka. *Proc Nat Acad Sci* 71(11):4503-4507, 1974.

Kirsten sarcoma virus (KiSV), murine leukemia virus (MuLV), Moloney sarcoma virus (M-MSV), endogenous rat type C virus (RaLV) and Rauscher murine leukemia virus (R-MuLV) RNA sequences were compared with normal rat cell RNA sequences. Cell RNA was prepared by the hot phenol method and the concentration was determined by the orcinol reaction. Viral ^{3}H-DNA was prepared by RNA-directed DNA polymerase reactions with RaLV, R-MuLV, M-MSV(MuLV), Ki-MuLV and 58-2T virus. The KiSV-specific ^{3}H-DNA (58-2TS DNA) was more than 80% hybridized with 58-2T cell RNA and NP cell (a BALB/3T3 cell transformed by KiSV) RNA while no hybridization was observed with R-MuLV RNA or BALB/3T3 RNA. 50-80S RNA was used for RNA subunit analysis. Increasing amounts of RNA were incubated with 500 cpm of ^{3}H-DNA and the hybridization was determined with single-strand specific S1 nuclease. Whole cell RNA absorbancy was measured at 260 nm and the RNA was precipitated in the presence of 50 μg/ml of yeast tRNA. RaLV ^{3}H-DNA transcript hybridized with increasing amounts of RaLV-producing cells, KiSV-transformed cells, normal rat cells, normal BALB 3T3 cells and Ki-MuLV-NIH/3T3 cells. About 20% hybridized with RNA of cells carrying the KiSV genome while none hybridized with RNA from Ki-MuLV-producing cells. KiSV-specific DNA did not hybridize with either M-MSV-transformed hamster cell RNA, R-MuLV or KiMuLV mouse cell RNA. Normal rat cells synthesize RNA hybridizable both to KiSV-specific and RaLV ^{3}H-DNA's. The high molecular weight RNA of ^{32}P-labeled RaLV was composed of 30 and 35S subunits. KiSV-specific ^{3}H-DNA hybridized only with the 30S fraction. It is suggested that a portion of the 30S KiSV genome RNA has sequences homologous to a portion of the RaLV genome. The 35S subunit is not found in mouse cells carrying the KiSV genome or in normal rat cells. 30S molecules are also found in non-producing cells transformed by M-MSV although these sequences have shown no relationship to KiSV by hybridization.

6791 EXPRESSION OF THE GENOMES OF TWO VIRUSES IN LYMPHOMATOUS TISSUE.

(Rus.) Zhdanov, V. M. (D. I. Ivanovskii Inst. Virol., Moscow, USSR), I. I. Terskikh, G. K. Zairov, S. A. Demidova, L. A. Monastyreva and Iu. I. Lorie. *Dokl Akad Nauk SSSR* 218(4):957-959, 1974.

Data were obtained on the expression of genomes of the oncornavirus and herpesvirus in the spleens of eight patients with lymphomatosis. Two types of DNA synthesis were present in the system under study; one associated with the RNA-template and, therefore, sensitive to ribonuclease and the other DNA-dependent one which was insensitive to ribonuclease. Products from the latter type of synthesis were heterogeneous and had sedimentation constants of 4-10 S. In the former type, the template remained intact and retained the sedimentation properties of high-molecular RNA (60-70 S) characteristic of oncornaviruses. RNA-dependent synthesis of DNA was observed in five cases and DNA-dependent synthesis in all eight cases. To isolate the virus-specific component of DNA-dependent synthesis of DNA, the nucleic acids from the cytoplasmic extracts were hybridized with DNA from cytomegalovirus. The results show that hybrids were formed both with cytomegalovirus DNA and DNA from human cells, but not with heterogenous calf thymus DNA. It is assumed that the DNA-dependent DNA product results from virus-induced and cellular synthesis. It is suggested that lymphomatous spleen cells contain two types of viruses: one of the *Herpes* group and the other possessing features of an oncornavirus.

6792 METACENTRIC CHROMOSOMES IN THE SPLEEN LINE KARYOTYPE FROM BALB/c MICE INFECTED WITH FRIEND AND RAUSCHER LEUKEMIA VIRUSES.

(Rus.) Ugriumov, E. P. (D. I. Ivanovskii Inst. Virol., Moscow, USSR), I. V. Dementiev and I. F. Barinskii. *Vopr Virusol* (1):49-54, 1974.

Cytogenetic and cytological studies of spleen lines from mice chronically infected with biologically active Friend and Rauscher leukemic viruses revealed the presence of metacentric chromosomes which are not characteristic of normal murine karyotype. Considering the character of the relationship between oncornaviruses and cellular genome, and also the similarity of nucleotide sequence in provirus DNA and in cellular DNA in the centromere region of the chromosomes, it is suggested that metacentric chromosomes are formed as a result of changes in the centromere areas of acrocentric chromosomes and their subsequent coalescence. The presence of metacentric chromosomes in the karyotype of murine lines may be taken as evidence of transformation by oncornaviruses.

6793 SUSCEPTIBILITY OF THE EMBRYONAL CELLS OF THE STRIPED HAIRY-FOOTED HAMSTER TO THE MUTAGENIC AND TRANSFORMING ACTION OF CERTAIN ONCOGENIC VIRUSES *IN VITRO*. *(Rus.)* Senina, E. T. (Inst. Exp. Clin. Oncol., Moscow, USSR) and E. E. Pogosiants. *Vopr Onkol* 20(5):38-46, 1974.

Studies were conducted to investigate the susceptibility of cell cultures of striped hairy-footed hamster (*Phodopus sungorus*) to the action of SA7(C8) adenovirus containing $10^{5.7}$ $TCID_{50}$/ml; SV40 virus (strain 128), containing $10^{7.5}$ $TCID_{50}$/ml; and cell-free extracts of Rous sarcoma virus (RSV) having an LD_{50} titer of 10^{-5}. In addition, morphological, biological and karyological changes in the cell lines as a result of the viral and spontaneous transformation were studied. It was demonstrated that embryonal hamster tissue cultures are readily transformed by the SA7(C8) monkey adenovirus, RSV and SV40. The SV40 virus-transformed cultures produced a continuous cell line, DET-1, with a hypodiploid relatively stable karyotype; the Rous virus-transformed cultures, a continuous line, DET-5, with unstable hypotriploid karyotype. The hamster cells *in vitro* showed a readiness to spontaneous transformation. The spontaneously transformed culture produced a continuous pseudodiploid heterogeneous line, DET-2. The embryonal hamster cell cultures showed little susceptibility to the mutagenic action of the viruses under study.

6794 INCORPORATION OF ^{14}C-2-DEOXY-D-GLUCOSE INTO THE LIPIDS OF NORMAL CELLS AS COMPARED TO VIRUS-TRANSFORMED CELLS. *(E.)* Steiner, M. R. (Baylor Coll. of Med., Houston, Texas), K. Somers and Sheldon Steiner. *Biochem Biophys Res Commun* 61(2):745-751, 1974.

The object of the study was to determine whether ^{14}C-labeled 2-deoxy-D-glucose (dglc) was a useful precursor for differentiation between glycolipid synthesis in nontransformed cells and cells transformed by either oncornavirus or herpes simplex virus. Cell lines used in the study were normal rat kidney (NRK); murine sarcoma virus-murine leukemia virus-transformed and producing NRK cells (NRK [MSV-MLV]); non-producing NRK cells with an MSV genome temperature sensitive for transformation; normal Wistar rat embryo cells (Wis); MLV-Wis, Wis cells chronically infected with and producing MLV; MSV-MLV transformed and producing Wis cells; herpes simplex type 2 transformed hamster cells; and normal hamster embryo fibroblast cells. The cells were grown in Eagle's medium containing ^{14}C-dglc. The lipids were extracted from them with chloroform-methanol and chromatographed on a silica gel plate. The results showed that the incorporation of dglc into the glycolipids of both DNA and RNA virus-transformed cells differed markedly from that of normal cells, and infected non-transformed cells. The percentage of labeled dglc lipids of transformed cells was markedly increased in the high and low mobility regions and markedly decreased in the intermediate region as compared to lipids of normal and infected cells. The results suggest that observed differences in dglc lipid patterns, of nontransformed *versus* transformed cells, are due to metabolic alteration rather than internal concentration of dglc. It is suggested that ^{14}C-dglc lipid patterns may provide useful markers for transformation.

6795 ULTRASTRUCTURAL STUDIES OF HUMAN ADENOVIRUS-PRODUCED RETINO-BLASTOMA-LIKE NEOPLASMS IN SPRAGUE-DAWLEY RATS. *(E.)* Mukai, N. (Retina Found., Boston, Mass.), S. Kobayashi and M. Oguri. *Invest Ophthalmol* 13(8):593-601, 1974.

Human adenovirus-induced autochthonous tumors of the retina were studied using electron microscopy to further elucidate the origin of human retinoblastoma. Human adenovirus type 12 was cultured in a HeLa cell monolayer. Newborn Sprague-Dawley rats (85) were each given a single intravitreous inoculation of 0.015-0.03 ml virus fluid ($10^{3.5}$-$10^{4.5}$ median tissue culture infectious dose HeLa cells/0.1 ml). Within 74-288 days after inoculation, a total of 16 rats developed intraocular tumors (18.8%). Massive intraocular tumor was observed in nine of these; the remaining seven rats showed microscopically detectable incipient tumors of the retina. Light microscopy showed tumor cell infiltration into the optic nerve head. A uniform histological appearance was also observed; incomplete rosettes associated with perivascular wreaths formed by medullo-epitheliomatous cells were common to all cases of massive growth and constituted the basic structural pattern of the tumors. Mitotic figures were frequent, particularly in those tumor cells forming incomplete rosettes. Clusters of bizarre ganglioneuronic tumor cells having no definite orientation and forming no rosettes were common, especially in areas between solid medullary tumor masses. Under electron microscopy, tumor cells forming incomplete rosettes appeared to have elongated, oval, or slightly indented nuclei with evenly distributed chromatin. No nuclear membrane abnormalities were found. Poorly differentiated intracytoplasmic organelles were noted. Few terminal bars and intracellular junction complexes were seen. Characteristic cilia, one cilium/cell, with a single pair of centrioles in the apical portion of the cytoplasm, were seen in many cells. All cilia possessed nine pairs of peripheral doublets with no central axis. Bizarre ganglionic tumor cells retained a much higher degree of differentiation. The broad rim of the cytoplasm showed more endoplasmic reticula, Golgi stacks, and mitochondria. The characteristic solitary cilia were also seen. Plasma cells were abundant throughout these specimens. Since human adenovirus type 12 is a potent oncogenic agent capable of producing, in animals, embryonal neurogenic tumors similar to the human, and since human retinoblastomas possess phenotypic similarities to the viral tumors (particularly the characteristic cilia), the viral origin of autochthonous human retinoblastomas seems a distinct possibility.

6796 ROLE OF DNA-DEPENDENT RNA POLYMERASES II AND III IN TRANSCRIPTION OF THE ADENOVIRUS GENOME LATE IN PRODUCTIVE INFECTION.

(E.) Weinmann, R. (Div. Biol. Biomed. Sci., Washington Univ., St. Louis, Mo.), H. J. Roskas and R. G. Rueder. *Proc Natl Acad Sci USA* 71(9):3426-3430, 1974.

DNA-dependent RNA polymerases I, II, III, obtained from cultured human (KB) cells infected with adenovirus 2 were studied to determine their role in transcription. The polymerases were purified by DEAE-Sephadex chromatography and incubated with α-amanitin at various concentrations to determine sensitivities. RNA polymerase II was inhibited by low concentrations (50% inhibition at 0.018 μg/ml). RNA polymerase III was inhibited at higher concentrations (50% inhibition at 19 μg/ml). RNA polymerase I was unaffected at concentrations up to 200 μg/ml. An inhibition curve was constructed for α-amanitin and found to be biphasic with inflection points at 0.02 and 20 μg/ml suggesting that RNA polymerases II and III are active in transcription in infected cells. Synthesis of 5.5S RNA and host 5S RNA showed the same sensitivity to α-amanitin as did RNA polymerase III suggesting that this enzyme is required for their synthesis. Synthesis of viral mRNA precursors demonstrated the same sensitivity to α-amanitin as did purified RNA polymerase II from infected KB cultured human cells. No RNA polymerase I activity could be demonstrated which suggests that host RNA polymerase I is not involved in transcription of the adenovirus genome at this time in infection. The specific effects of α-amanitin on different RNA polymerases indicates the requirement of RNA polymerases II and III for the transcription of adenovirus 2.

6797 INTRANUCLEAR SITE OF REPLICATION OF ADENOVIRUS DNA.

(E.) Simmons, T. (Yale Univ. Sch. Med., New Haven, Conn.), P. Heywood and L. D. Hodge. *J Mol Biol* 89(3):423-433, 1974.

Using electron microscope autoradiography, the intranuclear site of newly replicated adenovirus DNA was studied. HeLa S3 cells were maintained in suspension culture. Cells were arrested in the synthetic phase of the cell cycle by the technique of double thymidine blockage. Cells, released into thymidine-free medium after the second thymidine blockade, were infected with adenovirus type 2 at a concentration of 2000 viral particles/cell. In the population of infected cells, there were two separate periods of thymidine incorporation: one with a maximum at five hours post-synchronization and a second with a maximum at approximately 14 hours post-synchronization. Results indicated that continuing replication of viral DNA did not occur in association with the nuclear envelope. This occurred in the absence of the synthesis of host cell DNA. It was independent of the appearance of viral-induced changes in the nuclear morphology or variations in the length of radioactivity exposure. The results do not eliminate the possibility that a specific event, such as initiation of the synthesis of nucleic acid chains, occurs at unique sites on the nuclear envelope.

6798 STRUCTURAL PROTEINS OF ADENOVIRUSES. XI. PURIFICATION OF THREE LOW MOLECULAR WEIGHT VIRION PROTEINS OF ADENOVIRUS TYPE 2 AND THEIR SYNTHESIS DURING PRODUCTIVE INFECTION.

(E.) Everitt, E. (Wallenberg Lab., Uppsala Univ., Sweden) and L. Philipson. *Virology* 62(1):253-269, 1974.

Three low molecular weight structural proteins were purified from adenovirus type 2 virions. These proteins, which corresponded to polypeptides VI, VIII, and IX as defined by mobility in SDS-polyacrylamide gels, were purified to homogeneity by preparative polyacrylamide gel electrophoresis, gel exclusion, and ion exchange chromatography. The three proteins were immunologically distinct and did not cross react with the main capsid antigen or core proteins of adenovirus type 2 in immunodiffusion tests. Their amino acid compositions also differed from those of the major viral capsid and core proteins, and protein VI was rich in glycine (26%) and serine (22%). Pulse-chase experiments indicated that proteins VI and VIII may have been derived by cleavage from larger precursors, while protein IX appeared to be a primary translation product. Late in the productive infection of HeLa cells by adenovirus type 2, protein IX appeared to be synthesized in excess of the amount required for virus assembly. No excess pool of proteins VI and VIII was detected. The results indicate that the minor proteins VI, VIII, and IX are, or are derived from, unique species of polypeptides and are not generated by proteolytic cleavage of the large capsid proteins. These results together with previous reports also suggest that adenovirus type 2 contains at least ten polypeptides, the functions of which should be studied during macromolecular synthesis in virus-infected cells.

6799 SOMATIC CELL HYBRIDS BETWEEN MOUSE CELLS AND AVIAN SARCOMA VIRUS TRANSFORMED HAMSTER CELLS.

(E.) Hladka, M. (Cancer Res. Inst., Slovak Acad. Sci., Bratislava, Czechoslovakia) and C. Altaner. *Neoplasma* 21(6):629-637, 1974.

The properties of avian sarcoma virus-transformed hamster cell - A9 mouse cell hybrids were investigated. Transformed hamster cells, deficient in thymidine kinase, were fused with A9 cells, lacking hypoxanthine-guanine phosphoribosyl transferase, by means of UV-inactivated Sendai virus. The hybrids were of larger size and had a greater adhesive ability than either parent line. Chromosome count in the hybrid was under 80, while it was about 54 in the A9 and nearly diploid in hamster line. Unlike the parent hamster cell line, the hybrid clones did not produce tumors when injected into hamsters. Plating efficiency experiments showed the hybrids behaved as transformed cells and were not contact-inhibited. Experimental fusion of the hybrid with secondary chick embryo cells showed a need for a 1000-fold increase of cells for positive rescue of viral genome. This suppression of viral genome decreased slightly with the number of passage, as chromosomes were lost.

6800 DEVELOPMENT OF SYSTEMIC AND LOCAL TUMORS INDUCED BY AVIAN RETICULOENDOETHLIOSIS VIRUS AFTER THYMECTOMY OR BURSECTOMY. *(E.)* Linna, T. J. (Temple U. Health Sci. Ctr., Philadelphia, Pa.), C. Hu and K. D. Thompson. *J Natl Cancer Inst* 53(3): 847-853, 1974.

The effects of thymectomy and surgical and chemical (cyclophosphamide) bursectomy on tumor development and mortality were studied in Hy-Line chickens following local or systemic administration of avian reticuloendotheliosis (RE) virus, strain T. Significantly more thymectomized and bursectomized (surgically and chemically) chickens died from RE virus-induced tumors than did sham-operated and unoperated controls; the thymectomized and bursectomized birds also started dying earlier than the controls. Death from RE was also significantly more frequent in bursectomized birds given spleen cells from RE virus-infected histocompatible donors than in similarly treated controls. Locally induced tumors frequently regressed in the control birds, but most thymectomized and bursectomized birds developed progressively growing, ultimately fatal tumors. The data indicate that both thymus-dependent, cell-mediated immunity and the bursa-dependent, antibody-producing system have a host-protective influence — a "surveillance" function. The results are not compatible with a tumor-protective role for the antibody-producing system *in vivo*.

6801 BASE-SEQUENCE RELATIONSHIPS BETWEEN AVIAN RIBONUCLEIC ACID ENDOGENOUS AND SARCOMA VIRUSES ASSAYED BY COMPETITIVE RIBONUCLEIC ACID -- DEOXYRIBONUCLEIC ACID HYBRIDIZATION. *(E.)* Wright, S. E. (Univ. Washington Sch. Med., Seattle) and P. E. Neiman. *Biochemistry* 13(7):1549-1554, 1974.

Previous studies using RNA-DNA hybridization under conditions of DNA excess demonstrated that the majority of the genomic sequences of an endogenous RNA tumor virus of chickens (RAV-0) and a helper-independent, high oncogenic strain of Rous sarcoma virus (RSV) were present in low frequency (1-4 copies/haploid gennome) in the DNA of normal chicken cells and of wing-web tumor induced by RSV. A smaller fraction of the RSV genome was represented in normal chicken DNA. Competitive hybridization studies involving reactions between viral RNA and proviral DNA sequences in host cells were carried out to determine whether the sequences complementary to RSV in normal chicken cells were explained by the presence of the endogenous provirus, RAV-0 and to study the relationship between the RNAs from these two viruses. Results were compared to a theoretical model for competitive hybridization of RNA related to low-frequency genes based on conditions varying from a modest DNA excess to a modest RNA excess. Homologous unlabeled RNA and RAV-0 produced competitive effects in hybridization reactions between labeled RNA and related sequences in host cell DNA which conformed closely to theoretic competition curves and unlabeled RSV RNA competed in homologous reactions to about 90% of expected values. RNA from a second sarcoma-producing, helper-independent virus of different origin competed for RSV-related sequences as completely as did homologous RSV RNA. Competition experiments with RNA from different viruses revealed that RSV RNA competed for approximately 80% of sequences complementary to RAV-0 in normal chicken DNA and RAV-0 competed for approximately two-thirds of RSV-related sequences in wing-web tumor DNA. RAV-0 competed for a minimum of 83-90% of the sequences complementary to RSV in normal chicken DNA. It was concluded that the endogenous, nontransforming virus and the sarcoma viruses have extensive homology as well as measurable unshared genome segments. Further, almost all of the sequences complementary to RSV in normal chicken DNA are accounted for by the endogenous DNA provirus.

6802 MINOR BASE CHANGES IN TRANSFER RIBONUCLEIC ACID IN AVIAN MYELOBLASTOSIS VIRUS. *(E.)* Zamecnik, P. C. (Massachusetts Gen. Hosp., Boston), L. J. Rosenthal and K. Randerath. *Bibl Haematol* (39): 474-481, 1973.

To determine whether alterations in the minor base patterns of transfer RNA might be involved in viral oncogenesis, the minor base patterns of avian myeloblastosis (AMV) tRNA were compared with those of infected chick myeloblast and normal liver tRNAs. Significant differences in minor base content were found. The most striking difference was a 5- to 6-fold decrease in the percentage of 3-methylcytidine (m^3C) in the AMV tRNA as compared with the tRNA of the host cells. Significant decreases in 5-methylcytidine, ribothymidine, and dihydrouridine were also found in the AMV tRNA, as were significant increases in the percentages of 1-methylguanosine, N^2-methylguanosine, 7-methylguanosine, N^2,N^2-dimethylguanosine, and pseudouridine. Small but significant differences in the percentage composition of the major bases in the AMV and host cell tRNAs were also noted. The significant decrease in the concentration of m^3C in the AMV tRNA suggests that this base may be missing in certain virion tRNAs whose counterpart in the normal chick contains m^3C.

6803 VIRAL 70S RNA-DIRECTED DNA SYNTHESIS WITH A PURIFIED DNA POLYMERASE FROM HUMAN ACUTE LEUKEMIC CELLS. *(E.)* Gallo, R. C. (Natl. Cancer Inst., Bethesda, Md.), P. S. Sarin, M. G. Sarngadharan, M. S. Reitz, R. G. Smith and S. N. Bobrow. *Collect Pap Annu Symp Fundam Cancer Res* 25:477-493, 1974.

A purified DNA polymerase isolated from human acute leukemic cells accepts 70S RNA from avian myeloblastosis virus (AMV). This reaction is ribonuclease-sensitive and is stimulated 5-fold by oligo dT. Analysis shows that the product of the stimulated reaction is not covalently bound to the viral RNA template; in the absence of oligo dT, there was evidence of covalent bonding of the DNA product to the RNA. The DNA isolated from the hybrid is 4-6S in size, and it specifically hybridizes back to AMV 70S RNA. The product is not poly dT since there was no hybridization back to poly A. The leukemic enzyme favors dT_{12}·poly rA over dT_{12}·poly dA as a template, and is active with dG_{12}·poly rC. In short, it has the known essential biochemical properties of reverse transcriptase of RNA tumor viruses. It can be distinguished from the main normal human lymphocyte DNA polymerase by physical and biochemical properties, by acceptance of 70S RNA, and in particular by template preference.

6804 STRUCTURAL STUDIES OF AVIAN MYELOBLASTOSIS VIRUS: SELECTIVE RELEASE OF RIBONUCLEOPROTEIN POLYPEPTIDES FROM THE CORE COMPONENT AND PARTIAL PURIFICATION OF THE DNA POLYMERASE. *(E.)* Stromberg, K. (Natl. Inst. Health, Bethesda, Md.) and S. H. Wilson. *Biochim Biophys Acta* 361(1):53-58, 1974.

To facilitate study of avian myeloblastosis virus structural organization, a rapid single-step method is presented for fractionation of its core. Purified virus particles were mixed with 7.5% Sterox SL and then layered over a discontinuous sucrose gradient containing 0.1 to 1.0 M NaCl in the layer of 48% sucrose. In the 1.0 M NaCl, four polypeptides of apparent molecular weights of 91000, 64000, 34000, and 14000 were dissolved out, but two remaining core components of weights 98000, and 60000 remained in the centrifugation pellet. Subsitution of KCl, CsCl, Cs_2SO_4, and LiCl were less effective in the selective release of polypeptides, as were the lower concentrations of NaCl. Protein from the 1 M NaCl gradient had a 9.7-fold greater DNA polymerase activity than protein from the purified intact virus. The important advantage of the procedure is that the core is separated from possible contaminants before the release of the DNA polymerase. The total number of virion polypeptides is greatly reduced, all irrelevant high molecular weight polypeptides are removed, and the polypeptides of 64000 and 91000 molecular weight are left in 1 M NaCl solution.

6805 RIBONUCLEASE ACTIVITY TIGHTLY BOUND TO RIBOSOME-LIKE STRUCTURES ISOLATED FROM AVIAN MYELOBLASTOSIS VIRUS. *(E.)* Maly, A. (Czechoslovak Acad. Sci., Prague) and J. Riman. *Acta Virol (Praha)* 18(6):474-478, 1974.

The ribosome-like structures isolated from avian myeloblastosis virus (AMV) were studied. AMV was isolated from leukemic chicken plasma by centrifugation. The ribosome-like structures occurring in AMV were isolated from the AMV pellet by standard procedure. The results demonstrated that both viral ribosomes and host cell cytoplasmic ribosomes possessed an endonuclease activity that was detected by breaking down 60 S viral RNA to 4 S fragments. Both of the ribosomes were freed of ribonuclease by washing in 0.5 M NH_4Cl. Ribonuclease bound to ribosome-like structures and host cell cytoplasmic ribosomes seemed to differ by its mode of action from bovine pancreatic ribonuclease A, which split the 60 S viral RNA into fragments that were dissolved entirely in trichloroacetic acid. Therefore, ribosome-like structures were isolated from AMV and were shown to be firmly associated with the ribonuclease. This was also the case with host cell cytoplasmic ribosomes. The function of ribosomal ribonuclease is still unknown.

6806 ISOLATION FROM BALB/c MOUSE CELLS OF A STRUCTURAL POLYPEPTIDE OF A THIRD ENDOGENOUS TYPE C VIRUS. *(E.)* Stephenson, J. R. (Natl. Cancer Inst. Bethesda, Md.), S. R. Tronick and S. A. Aaronson. *Cell* 3(4):347-353, 1974.

A 12,000 molecular weight type C viral polypeptide, p12, was isolated from BALB/c mouse cells. Homogenates of BALB/c mouse liver or tissue culture cells were treated with 2 μg/ml bovine pancreatic deoxyribonuclease for four hr at 4 C. One ng ^{125}I-p12 R-murine leukemia virus was mixed with approximately 2000 mg BALB/c liver cell extract. The mixture was then subjected to sequential purification procedures involving acid precipitation, ion exchange chromatography, and agarose gel filtration in the presence of 6 M guanidine hydrochloride. At each step, recovery of the ^{125}I-labeled p12 marker was compared with that of total cell protein and immunologic reactivity in the group-specific immunoassay for p12. The increase in specific activities of ^{125}I-labeled p12 and cellular p12 antigen were comparable at each step of purification, indicating that the immunologic reactivity purified from BALB/c cells was associated with a polypeptide biochemically similar to the type C viral p12 polypeptide. The p12 isolated from BALB/c cells was tested in three different p12 immunoassays and was shown to be distinct from the corresponding polypeptides of two previously described endogenous viruses of BALB/c cells but indistinguishable from that of a xenotropic virus endogenous to NIH Swiss cells. The expression of endogenous viruses containing each of the three distinguishable p12 antigens was differentially affected by two classes of chemical inducers, halogenated pyrimidines and inhibitors of protein synthesis. These findings provide evidence for the existence of genetic information of three distinguishable endogenous viruses within cells of the BALB/c mouse strain.

6807 EVOLUTION OF C-TYPE VIRAL GENES: INHERITANCE OF EXOGENOUSLY ACQUIRED VIRAL GENES. *(E.)* Benveniste, R. E. (Natl. Cancer Inst., Bethesda, Md.) and G. J. Todaro. *Nature* 252(5483):456-459, 1974.

A scheme for viral transmission among species is proposed. Hybridization of unique sequence domestic cat cellular ^{3}H-DNA (1.8×10^7 cpm/μg) with the cellular DNA of other Felidae (European wildcat, jungle cat, leopard cat, tiger and lion) showed that they are all closely related. Hybridization of RD114 C-type viral ^{3}H-DBA with cat cellular DNA's showed that homology was greatest with domestic cat DNA. Only four species of *Felis* contained RD114 related sequences. Nucleic acid sequences related to RD114 were found in six species of Old World monekys.

Thermal stability of the hybrids formed between baboon viral ^{3}H-DNA and various feline and primate cellular DNA's resulted in lower thermal melting points with the Old World monkey DNA's (approximately 11 C lower than the homologous hybrid). This index of base-pair mismatching between the strands of a double-strand DNA molecule suggests that the overall unique-sequence cellular DNA's of the Old World monkeys are heterologous with respect to the cat species. Since RD114-related nucleic acid sequences can be found in all Old World monkey and ape tissues but in only four Felidae species it is proposed that RD114 is a primate C-type virus that infected an ancestor of the domestic cat. Evidence presented suggests that C-type viral gene was transmitted horizontally and subsequently perpetuated through the germ line.

6808 STUDIES ON A HUMAN CELL LINE (ESP-1) PRODUCING TYPE C VIRUS PARTICLES. *(E.)*

Priori, E. S. (Texas Med. Cent., Houston), L. Dmochowski, B. Myers, T. Shigematsu and J. R. Wilbur. *Bibl Haematol* (39):720-731, 1973.

A culture of human origin (ESP-1) has been established from the pleural effusion cells of a 5.5-year-old child with Burkitt's lymphoma. From the tenth tissue culture passage on, the ESP-1 cells produced budding, immature, and mature virus particles resembling the type C particles of murine leukemia. No type C particles were seen in the first passage or in the original pleural effusion cells. Varicella virus particles were found at autopsy in the cells of the liver, pancreas, and adrenal glands, but not in the cultured pleural effusion cells. The C type virus particle yield in the cultured cells was augmented by the addition of 2% dimethylsulfoxide or co-culture of ESP-1 cells with whole human embryo cells. The ESP-1 cells were definitely of human origin and they bore phenotypic and genotypic similarities to the surface leukocytes of the patient's parents. The cell culture became infected with mycoplasma sometime after the thirteenth passage; attempts to eradicate the mycoplasma have been unsuccessful. Cells of the ESP-1 culture gave cytoplasmic fluorescence in the fixed indirect immunofluorescence test with sera from patients with Burkitt's lymphoma or other solid tumors (particularly lymphoma, osteosarcoma, rhabdomyosarcoma, and reticulum cell sarcoma); positive reactions were also obtained with sera from normal donors with high titers to Epstein-Barr virus (EBV). Only a small number of sera from normal donors without high titers to EBV reacted at low titer. The ESP-1 particles contained the group specific antigenic determinant gs-3, which is shared among the known mammalian leukemia viruses; they did not contain the gs-1 mouse antigenic determinant. The data indicate that the ESP-1 virus may share some common antigenicity with the other known type C viruses of animal leukemia and sarcoma. The possible immunologic relationship between Burkitt's lymphoma and other solid tumors indicated by the data remains to be determined.

6809 RADIOIMMUNOASSAY FOR THE MAJOR STRUCTURAL PROTEIN OF HAMSTER TYPE C VIRUSES. *(E.)*

Charman, H. P. (Dep. Pathol., Univ. South. California, Los Angeles), N. Kim and R. V. Gilden. *J Virol* 14(4): 910-917, 1974.

A radioimmunoassay (RIA) for the major group-specific antigen (p30) of purified hamster type C viruses was developed. It was based on the Farr technique and involved the use of ^{125}I-labeled antigen, guinea pig (or goat) antiserum to the antigen, and goat anti-guinea pig (or rabbit anti-goat) gamma globulin. The assay detected as little as 5 ng/ml of the p30 antigen and was 35- to 70-fold more sensitive than the complement-fixation test for the same antigen. When Graffi hamster virus, B-34 virus, and a virus isolated from a hamster tumor induced by Gross virus (the last two being mouse-hamster pseudotypes) were compared in homologous and heterologous intraspecies assays, no evidence of type specificity for these proteins was noticed. The pseudotype viruses showed no evidence of mouse virus p30 antigenic determinants. An interspecies antigen assay employing ^{125}I-labeled hamster p30 and anti-feline p30 was completely inhibited by cat (feline leukemia virus), hamster, and rat virus, to a slightly lesser degree by mouse viruses, and poorly inhibited (15-30%) by RD 114 and Gibbon ape viruses. The Mason-Pfizer virus did not inhibit this assay. Hamster p30 was detected by the RIA in individual embryos from two LSH hamsters and in several adult tissues, excluding muscle, at levels below that required for detection in complement-fixation tests.

6810 VIRAL ENVELOPE ANTIGENS OF THE VIRUSES OF FELINE LEUKEMIA-SARCOMA COMPLEX. *(E.)*

Sarma, P. S. (Natl. Cancer Inst., Bethesda, Md.) and T. Log. *Bibl Haematol* 39:113-124, 1973.

The occurrence of distinct viral envelope antigens in the feline C-type viruses and the occurrence of corresponding cell receptor sites in feline embryo cells was investigated by viral neutralization and interference tests. Viral interference in feline leukemia and sarcoma viruses followed three patterns which appeared to be related to envelope antigenic relationships between the interfering and challenge viruses. Based on these patterns, the feline C-type viruses were classified into three subgroups of A, B and C. Feline leukemia viruses of the A subgroup induced resistance to challenge with focus-forming feline leukemia psuedotypes of murine sarcoma virus of the same subgroup but not to viruses of subgroups B and C. The presently known naturally occurring feline leukemia viruses of the B and C groups not only interfered with the homologous subgroup challenge viruses, but also with viruses of the A subgroup. This interesting pattern of viral interference was established as being due to the presence in the B and C subgroup leukemia virus stocks of subgroup A virus. Purification of the B and C subgroup viruses was achieved by viral cloning techniques with and without the use of virus neutralizing antisera against the A subgroup. Serum neutralizing tests showed that the viral envelope antigens responsible for viral interference were the same as those involved in the induction of virus neutralizing antibodies. These results further confirm the presence in uncloned B and C viruses of the major envelope antigen of the A subgroup virus. The removal of this A component virus from B and C virus stocks by A antiserum treatment suggests that the envelope antigens of A, B and C are distinct and can be isolated as pure types.

6811 GLYCOLYSIS DURING EARLY INFECTION OF FELINE AND HUMAN CELLS WITH FELINE LEUKEMIA VIRUS. *(E.)* Bardell, D. (Harvard Sch. Public Health, Boston, Mass.) and M. Essex. *Infect Immun* 9(5):824-827, 1974.

An investigation was carried out on glucose uptake and lactic acid production in cell cultures following inoculation with feline leukemia virus (FeLV). To test the effects in cells of different types and species, a secondary culture of fetal cat lung fibroblasts (FLF-3) and an established line of human epithelioid carcinoma (HEp-2) were used. They were grown on MEM containing 10% fetal calf serum. In some experiments, FLF-3 cells were maintained in a stationary phase by use of MEM containing 0.5% fetal calf serum. Assays for infectivity of cells were carried out by indirect immunofluorescence test for cytoplasmic FeLV gs antigen. At 12-hr intervals, the medium was decanted from inoculated and uninoculated cultures, centrifuged to remove suspended cells, and analyzed for glucose and lactic acid content. Fresh medium was then added to the cultures. At 48 hr after inoculation, 50% of the cells were found to be infected, but their morphology was unaltered and infection did not affect the growth rate as determined by cell count. Increased glucose uptake and concomitant increased production of lactic acid were observed for infected cells of both the FLF-3 and HEp-2 cultures, compared to corresponding uninfected cells. For example, glucose uptake at 48 hr was 15.22 μmol/10^6 cells for infected FLF-3 cells compared to 11.56 μmol/10^6 cells for corresponding uninfected controls, while lactic acid production was 17.63 μmol/10^6 cells for infected cells compared to 12.76 μmol/10^6 cells for uninfected controls. For FLF-3 cells maintained in stationary phase, however, there were no differences in glucose uptake and lactic acid production between uninfected and infected cells. The results demonstrated that infection with FeLV induces enhanced glycolysis in cells from homologous and heterologous species with respect to the natural host of the virus, and also in cells of epithelioid and fibroblastic morphology.

6812 ULTRASTRUCTURAL ANALYSIS OF HERPES-TYPE VIRUSES. *(E.)* Heine, U. I. (Natl. Cancer Inst., Bethesda, Md.) and A. J. Dalton. *Collect Pap Annu Symp Fundam Cancer Res* 25:63-93, 1974.

Comparative morphological investigations using the electron microscope were made of cells infected with herpes viruses belonging to group A (herpes simplex, pseudorabies), or to group B (cytomegalo-, varicella-zoster, Epstein-Barr, herpes saimiri, herpes sylvilagus, Marek's disease, and Lucké frog kidney tumor). This classification is based on the greater infectiousness of the former group, the greater cell-association of the latter. Most studies were carried out with cells infected *in vitro*. For infections with viruses of group A, the early cellular response consisted of peripheral condensation and increased electron density of the nuclear chromatin, extreme proliferation of the nuclear membrane, and, later, accumulation of naked virions in the nucleus. Envelopment of immature virions occurred through budding at either the nuclear or the cytoplasmic membrane. After release into extracellular spaces, the virions were characterized by a dense viral core, a capsid of relatively low electron density, and an outer envelope composed of a unit membrane. The components of the virions were easily distinguishable because of an electronlucent area between capsid and envelope. For infections with viruses of group B, the host cell reaction was slower but in many respects was similar to that for group A viruses. However, individual differences among the group B viruses suggested three subgroups. The first, which included the classical cytomegalovirus, was characterized by nuclear and cytoplasmic inclusion bodies in the infected cells and by a small number of mature and uniform virions in extracellular spaces. The second, which was represented by Marek's disease, herpes saimiri, herpes sylvilagus, and Lucké frog tumor viruses, also produced nuclear and cytoplasmic inclusion bodies in infected cells, but possessed uniform virions only inside the nucleus, while enveloped particles in the cytoplasm and in extracellular spaces were highly pleomorphic. The third subgroup, represented by Epstein-Barr virus, had morphological features closely resembling herpes simplex virus. The electron microscopic observation that group B viruses may shed large numbers of mature, enveloped virions into extracellular spaces, leads to a questioning of the meaning of the term, cell-association. The possibility is suggested that the extracellular viruses of group B are noninfectious, and that intra-cellular virions, either within nuclear vacuoles or budding from nuclear membranes, are the infectious particles. It is concluded that there are no morphological criteria which can be related to the apparent close cell association of group B viruses.

6813 TYPING OF HERPES SIMPLEX VIRUS STRAINS OF GENITAL AND NONGENITAL ORIGINS. *(E.)* Kawana, T. (Dep. Obstet. Gynecol., Univ. Tokyo Sch. Med., Japan) and K. Yoshino. *Jpn J Microbiol* 18(3): 235-241, 1974.

This study was to investigate the occurrence of Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) in genital infections of Japanese patients, and also to determine whether the chick embryo (CE) cell plaque marker technique is feasible as a simpler method of type determination than the cumbersome serological methods.

Type determination was the neutralization technique of Hamper, which uses type-specific hyperimmune rabbit IgM and complement; typing was unequivocal. Previous studies using standard methods had given intermediate values between HSV-1 and HSV-2 for many samples, due to cross reactions. Herpes cultures were obtained from the genital areas of 20 females and seven males. The results showed that one third of all the genital isolates were type 1, previously believed to be associated with non-genital areas. In nongenital infections of nine adults, eight produced HSV-1 isolates and two neonates with acute generalized infections had HSV-2, probably due to the presence of maternal lesions. The new findings were partly ascribed to the elimination of intermediate types. The genital HSV-1 strains were from females, mostly from acute infections, implying that either HSV-2 causes chronic genital infections, or that the HSV-1 in acute genital infections remains *in situ* in a latent form which somehow undergoes a type change to HSV-2. These observations may have relevence to the previously reported close relationship between HSV-2 and cervical cancer. The CE plaque marker technique of Figueroa and Rawls and Lawry *et al.* was found to be successful in identification of HSV types, HSV-2 being associated with markedly larger plaques than HSV-1.

6814 ANTIBODIES TO HERPESVIRUS TYPES 1 AND 2 AND CERVICAL CANCER.

(E.) Rawls, W. E. (Baylor Coll. Med., Houston, Tex.), E. Adam, J. W. Smith and J. L. Melnick. *Collect Pap Annu Symp Fundam Cancer Res* 25:574-583, 1974.

Reliability of the assay for antibody to herpesvirus type 2 is critical to evidence for correlation between cervical cancer and the occurrence of such antibodies in the serum of affected subjects. Herpesviruses types 1 and 2 are not, however, antigenically distinct, but possess common, as well as specific, antigens. Criteria for the presence or absence of antibodies to herpesvirus type 2 were previously based on the assumption that immune responses to type 1 and type 2 viruses were independent. Using the ^{51}Cr release test to quantitate antibody activity to antigens which develop at the surface of cultured BHK-21 cells infected with type 1 or type 2 herpesvirus, it was shown that all reactivity against either type 1 or type 2 infected cells was removed by absorption of type 1 serum with unlabeled type 1-infected cells, but that while absorption of type 2 serum with unlabeled type 1-infected cells removed all of the activity of the serum to labeled type 1-infected cells, it only reduced the activity against type 2-infected cells by about 1/2. The activity remaining after absorption with type 1-infected cells thus appeared to be specific for type 2-specific antigen. Infection of persons with herpesvirus type 2 without prior infection with herpesvirus type 1 was found to result in a high serum level of cytolytic activity against the specific antigen. If the type 2 infection followed a type 1 infection, low serum levels of activity, or no activity, developed against the specific antigen. These findings showed that the antibody response to type 2 antigen detectable by the ^{51}Cr release test was dependent upon the prior experience of the person with regard to type 1 virus. Thus, the immune responses to the two types of herpes virus do not appear to be independent events. When this conclusion was taken into consideration in analyses of antibody levels among women with cervical cancer and control women, uniform differences in the occurrence of antibody activity to type 2 virus between cases and controls were found in segments of the population studied. The differences between cases and controls could not be accounted for by differences in sexual promiscuity. It was concluded that a causal relation between herpesvirus type 2 and cervical cancer remains a tenable hypothesis to explain the observed association between the virus and the cancer.

6815 REGULATION OF HEMOGLOBIN SYNTHESIS IN A MURINE ERYTHROBLASTIC LEUKEMIC CELL: THE REQUIREMENT FOR REPLICATION TO INDUCE HEMAGLOBIN SYNTHESIS.

(E.) McClintock, P. R. (Univ. Tennessee-Oak Ridge Grad. Sch. Biomed.-Sci.) and J. Papaconstantinou. *Proc Natl Acad Sci USA* 71(11):4551-4555, 1974.

Friend virus-infected leukemic cells derived from DBA/2J mice were placed into complete medium (supplemented with 15% fetal calf serum) with or without Me_2SO (2% volume/volume culture medium) to determine the time course of hemoglobin accumulation. Hemoglobin was measured spectrophotometrically at 410 nm and showed negligible amounts in the absence of Me_2SO. Cultures seeded at low densities (1.5 x 10^5 cells/cm^3) with Me_2SO required two generation periods (48 hr) before hemoglobin synthesis was initiated. Cultures seeded at moderately high densities (4.2 x 10^5 cell/cm^3) also required two generation periods before hemoglobin synthesis occurred. Cultures seeded at high densities (37 x 10^5 cell/cm^3) failed to replicate and showed no hemoglobin synthesis. When Friend leukemic cells were supplemented with 7.5% or 2.5% fetal calf serum the generation time was increased to 34 hr and 48 hr, resp. The induction time of hemoglobin by Me_2SO was lengthened to 72 hr and 96 hr corresponding to two generations. $N^6,O^{2\prime}$-dibutyryladenosine 3′:5′-cyclic monophosphate [Bt_2cAMP (0.5 mM)] was added to Me_2SO-treated Friend leukemic cells at the beginning of the culture period and completely inhibited cellular replication and hemoglobin synthesis. Bt_2cAMP (1.0 mM) added to cultures 24 hr after addition of Me_2SO extended the generation time from 24 hr to 38 hr and delayed hemoglobin synthesis to 78 hr. Bt_2cAMP (1.0 mM) added to cultures 48 hr after addition of Me_2SO had no effect on the appearance of hemoglobin although further cellular replication was inhibited. Friend leukemia cells were arrested at the G1 phase by seeding in isoleucine-deficient medium for 40 hr. The cells were released from the block by the addition of isoleucine-containing medium, and Me_2SO was added; hemoglobin appeared 47 hr later. The cells had gone through two mitoses prior to the appearance of hemoglobin. These results support the hypothesis that Friend leukemia cells require two generations of DNA synthesis and/or mitosis after the addition of Me_2SO for the induction of hemoglobin.

6816 THE EFFECT OF HYDROCORTISONE AND BROMODEOXYURIDINE (BUdR) ON THE EPSTEIN-BARR HERPES VIRUS IN HUMAN LYMPHOBLASTOID CELL LINES.

(E.) Joncas, J. G. (Inst. Microbiol. Hyg. Montreal, Ville Laval, Canada). *Rev Can Biol* 33(2):135-147, 1974.

Studies defining the effect of hydrocortisone and bromodeoxyuridine (BUdR) on Epstein-Barr virus (EBV)-infected murine and human lymphoblastoid cell lines are reviewed. Although the EBV genome is present in most human lymphoblastoid cell lines, its expression varies considerably, ranging from a complete lytic productive cycle to the expression only of a soluble complement fixing or nuclear antigen. Exposure of EBV-carrying cells to thymidine analogues such as BUdR, cocultivation with other cells, starvation, hypothermia, and cyclic AMP generally result in more complete expression of the viral genome by an unknown mechanism. BUdR may exert its action by derepression of a cellular or viral thymidine kinase. Using hydrocortisone in lymphoblastoid cell lines and BUdR in the Raji cell line, it was found that cell viability was affected in certain cell lines but not in others. In the affected cell lines, the products of the lytic cycle of the virus (early antigen, virus capsid antigen and viral particles) increased concomitantly. After a delayed and sluggish interferon response, cell viability and viral genome expression returned to their pre-activation status. In the unaffected cell lines, there was a brisk interferon response which appeared to prevent detectable activation of the viral genome. These results suggest that a kinetic relationship exists between viral genome expression, at least in the lytic productive cycle, and interferon synthesis. The EBV carrying lymphoblastoid cell line might serve as a model for study of the intracellular control of persistent viral infections and individual variations in the quality of the interferon response.

6817 CELL RELATIONSHIPS IN TRANSFORMATION OF HUMAN LEUKOCYTES BY EPSTEIN-BARR VIRUS. *(E.)*

Pope, J. H. (Queensland Inst. Med. Res., Brisbane, Australia), W. Scott amd D. J. Moss. *Int J Cancer* 14(1):122-129, 1974.

Transformation of human leukocytes by the QIMR-WIL strain of Epstein-Barr (EB) virus *in vitro* involved cooperation between adherent cells (probably macrophages) and nonadherent cells (predominantly lymphocytes). Adherent cell cultures showed no signs of transformation 19 days after inoculation of EB virus, while nonadherent cell cultures showed a large number of degenerate cells and small clusters of active transformed cells. In a combined culture of adherent and nonadherent cells, numerous large aggregates of actively proliferating cells were present 19 days after virus inoculation. Study of the inhibition of cell populations by mitomycin C and identification of cells by sex chromosomes showed that the cells proliferating in response to EB virus infection were present in the nonadherent rather than the adherent cell population. Unlike macrophages, autochthonous fibroblasts did not enhance proliferation when cultured with nonadherent cells, but were apparently inhibitory. Investigation of virus-cell relationships in transformation indicated that inoculation of the nonadherent cell population was essential for EB viral transformation; inoculation of macrophages only was insufficient. The data indicate that infection of nonadherent cells with QIMR-WIL EB virus induces some cells to proliferate, and that the enhancing role of the macrophages does not involve infection with virus.

6818 EB VIRUS-ASSOCIATED MACROMOLECULES IN CELLS DERIVED FROM HUMAN TUMORS. *(E.)*

zur Hausen, H. (Univ. Wurzburg Inst. Virol., Germany), V. Diehl, H. Wolfe and H. Schulte-Holthausen. *Collect Pap Annu Symp Fundam Cancer Res* 25:517-530, 1974.

Previous evidence supporting the hypothesis that Epstein-Barr virus (EBV) represents a tumor virus and plays a causal role in Burkitt's tumor and anaplastic carcinomas of the pharynx include the relatively high antibody titers against EBV in sera of subjects with these diseases, the stimulation of lymphoproliferation *in vitro* by EBV, the relative ease of culture of lymphoblastoid cell lines from peripheral blood or lymphatic tissues of donors with EBV antibodies, and analogies with oncogenic animal herpes virus systems (Marek's disease herpes virus and herpes saimiri). The present additional evidence includes homology of sheared EBV-DNA with DNA derived from human lymphoblastic lines (Raji cells) and human tumor biopsy cells (Burkitt's tumor and nasopharyngeal carcinoma), hybridization between EBV-specific complementary RNA (obtained by transcription of EBV-DNA *in vitro* with the aid of *E. coli* RNA polymerase) and DNA from Raji cells or tumor biopsy cells, hybridization between complementary RNA and EBV-DNA *in situ* in heat-denatured EBV-synthesizing P3HR-1 cells, using autoradiography, and reinfection of human lymphoblastoid cell lines (Raji and S95) with EBV, resulting from special UV treatment of the virus which causes a delay in the production of early antigen. Infectious virus was recovered from at least three of seven lines found to be refractory under usual conditions. The results of the reinfection studies were interpreted to indicate that EBV synthesis in human lymphoblastoid cell lines results from induction of endogenous viral genomes and not from spreading of the virus by infection.

6819 PROGRAMMING OF EVENTS IN EPSTEIN-BARR VIRUS-ACTIVATED CELLS INDUCED BY 5-IODODEOXYURIDINE. *(E.)*

Hampar, B. (Natl. Cancer Inst., Bethesda, Md.), J. G. Derge, M. Nonoyama, S. Y. Chang, M. A. Tagamets and S. D. Showalter. *Virology* 62(1):71-89, 1974.

Programming of the Epstein-Barr (EB) virus productive cycle was studied in synchronized nonproducer Raji cells and producer EB-3 cells after activation by 5-iododeoxyuridine (IdU, added for 60 min during the S-1 period of the cell cycle). The activated Raji cells synthesized only the virus associated early antigen (EA) complex, while some activated EB-3 cells also synthesized virus structural antigens (VCA). Synthesis of EA corresponded to the early G-2 period of the cell cycle, and DNA synthesis after removal of the IdU was not required for the subsequent synthesis of EA. The time required for EA synthesis, including associated RNA and protein requirements, was the same in cells allowed to complete the S phase and in cells in which DNA synthesis was inhibited by 1-B-D-arabino furanosylcytosine (ara-C) or hydroxyurea (HU) (added after the removal of IdU). Thus, the synthesis of EA appeared to be independent of the cells' S phase, and a possible association between EA synthesis and the synthesis of cellular G-2 proteins is suggested. The synthesis of EA in abortively infected Raji cells was followed by the prolonged incorporating of low levels of deoxycytidine. At least some of the EA-positive cells were also able to reach mitosis, indicating that the synthesis of EA did not result in immediate cell death. The synthesis of VCA occurred 3-4 hr after the synthesis of EA in approximately 50% of the activated EB-3 cells. VCA synthesis required a short period of DNA synthesis following EA synthesis. The inability of about 50% of the activated EB-3 cells to synthesize VCA was ascribed to the toxic effects of the incorporated IdU. Those EB-3 cells which did progress to VCA synthesis showed productive replication of viral DNA which continued for several hours after the initial appearance of VCA.

6820 THE DISTRIBUTION OF 5-BROMODEOXYURIDINE IN THE DNA OF POLYOMA-TRANSFORMED MOUSE CELLS AND SOME APPARENT EFFECTS ON TRANSCRIPTION. *(E.)*

Grady, L. J. (New York State Dep. Health, Albany) and W. P. Campbell. *Exp Cell Res* 87(1):127-131, 1974.

The DNA of polyoma-transformed mouse cells grown in the presence of 5-bromodeoxyuridine (BUdR) was examined in neutral and alkaline CsCl gradients. None of the DNA from the cells grown in BUdR banded at the density of normal mouse DNA in a neutral CsCl gradient, indicating that some of the thymidine in all of the cells was replaced by the analog. The banding pattern of the BUdR-containing DNA in alkaline CsCl gradients was quite broad, indicating considerable heterogeneity in density and, hence, in BUdR substitution, throughout the genome. The DNA of BUdR-treated cells was labeled with radioactive BUdR, while that of control cells was labeled with radioactive thymidine. The distribution of tritium between repetitive and nonrepetitive sequences was identical in both cases. Thus BUdR replaced thymidine to the same extent in both categories of DNA. The transcription of nonrepetitive DNA in cells grown in 3 μg/ml BUdR for 10 passages was studied by RNA-DNA saturation hybridization experiments. The extent of nonrepetitive DNA expression was similar in confluent and subconfluent cells. Assuming that only one strand of the mouse genome was transcribed, 25% of the nonrepetitive DNA that could be transcribed actually was transcribed in these cells. Therefore, one effect of BUdR incorporation into the DNA is an apparent reduction in the amount of nonrepetitive DNA transcribed.

6821 CORRELATION BETWEEN EBV INFECTIONS AND MALIGNANT LYMPHOMAS. *(E.)*

Schmitz, H. (No affiliation). *Recent Results Cancer Res* 46:55-58, 1974.

The Epstein-Barr Virus (EBV) has been identified with great regularity in human Burkitt tumors but it cannot be detected in transformed tumor cells *in vivo*. There are three indications for the etiological involvement of EBV in the Burkitt tumor: 1. identification of EBV-transformed lymphoblastoid tumor cells, which mostly contain viral nucleic acid; 2. the fact that all tumor patients have EBV antibodies; 3. it is primarily children who may have had no previous EBV infection who develop Burkitt tumors. To determine the exact point of EBV infection in lymphoma patients, EBV IgM antibodies were examined by immunofluorescence. These IgM antibodies can only be detected in the sera of patients who have been infected with EBV for only a few weeks. As the acute infection regresses and the virus is eliminated from the blood, the EBV IgM antibodies also disappear. In cases with infectious mononucleosis, there was a rapid decrease in EBV IgM antibodies so that the presence of EBV IgM antibodies indicated a recent infection. Similar studies were attempted with Burkitt tumors and Hodgkins disease, but due to the need for cases with recent origins, results with these diseases were not conclusive.

6822 A STUDY ON POLYOMA- AND SV40-INDUCED CHROMOSOME REPLICATION AND MITOSIS. *(E.)*

Weil, R. (Dep. Mol. Biol., Univ. Geneva, Switzerland), E. May, P. May and H. Turler. *Collect Pap Annu Symp Fundam Cancer Res* 25:397-419, 1974.

Lytic infection with polyoma virus in contact-inhibited, primary kidney cell cultures was studied on the molecular and cytological level. Subsequently, the lytic infection was compared with the abortive infection induced by simian virus 40 (SV40) in the same tissue culture system. Results showed that the sequence of early events during the lytic and abortive infection is analogous and leads in both instances to virus-induced chromosome replication. The sequence is characterized by: transcription of early viral RNA, appearance of virus-specific (intranuclear) T-antigen, a lag period between the increase in the number of T-antigen-positive nuclei and the increase in the number of DNA-synthesizing cells, and, finally, chromosome replication. The lag is suggested to involve an activation of the cellular DNA-synthesizing apparatus, mediated by a temperature-sensitive metabolic process which is referred to as a "psychosensitive event." In the lytic infection with polyoma virus, chromosome replication is accompanied by the production of progeny virus and is followed by cell lysis but not by mitosis. In the abortive infection with SV40, chromosome replication is followed by mitosis while essentially no viral DNA or capsid proteins are produced. These results suggest that polyoma and SV40 (and possibly the related papilloma viruses) act primarily as mitogens and that their mitogenic action is mediated by an activator specified by the viral genome.

6823 RECIPROCAL INHIBITION OF MOUSE LEUKEMIA VIRUS INFECTION BY *Fv-1* ALLELE CELL EXTRACTS. *(E.)* Tennant, R. W. (Biol. Div., Oak Ridge Natl. Lab., Tenn.), B. Schluter, W. K. Yang and A. Brown. *Proc Natl Acad Sci USA* 71(10):4241-4245, 1974.

Soluble extracts of mouse cells with $Fv\text{-}1^n$ or $Fv\text{-}1^b$ gene alleles specifically and reciprocally inhibit the infection of B- or N-tropic mouse leukemia viruses in permissive cell cultures. NB-tropic virus infection was not inhibited by either cell extract. Extracts from $Fv\text{-}1^-$ cells did not inhibit infection by the three virus host-range types, but N- or B-tropic virus infection of $Fv\text{-}1^-$ cells was inhibited by extracts of the nonpermissive cells, and $Fv\text{-}1^{nb}$ cell extracts inhibited both viruses. The maximum degree of inhibition was 50-80% as determined by immunofluorescence and plaque assays, with extracts containing up to 500 µg/ml of nonpermissive cell protein. The inhibitor(s) was relatively unstable in that its activity was lost after two hr at 37 C or 30 min at 56 C. The inhibitor(s) was most effective if added two hr before or within two hr after infection; it did not react directly with the virus, inhibit virus attachment, or inhibit the normal cell functions. The data indicate that nonpermissive mouse cells contain a product which may be determined by the *Fv-1* gene and which inhibits some early post-penetration event(s) in leukemia virus infection.

6824 INCREASED COLLAGEN SYNTHESIS IN KIRSTEN SARCOMA VIRUS-TRANSFORMED BALB 3T3 CELLS GROWN IN THE PRESENCE OF DIBUTYRYL CYCLIC AMP. *(E.)* Peterkofsky, B. (Natl. Cancer Inst., Bethesda, Md.) and W. B. Prather. *Cell* 3(3):291-299, 1974.

The effects of dibutyryl cyclic AMP (dbcAMP) on the incorporation of ^{14}C-proline into the collagen polypeptide chain of a Kirsten sarcoma virus-transformed line derived from BALB 3T3 (Ki-3T3) were studied. Growth of the Ki-3T3 cells in the presence of dbcAMP resulted in an alteration in morphology, inhibition of growth, and increased collagen synthesis as measured by the incorporation of ^{14}C-proline into collagenase-digestible protein. There was an increase in the incorporation of ^{14}C-proline into collagen not only when expressed as dpm/µg DNA or protein, but also when expressed as the relative rate of collagen synthesis compared to total cellular protein synthesis; this suggests that an alteration in amino acid transport cannot fully account for the increased incorporating into collagen. The cell characteristics studied were all affected over a range of concentration from 0.10-1.0 mM dbcAMP, but each had a slightly different dose-response curve. At 0.5 mM dbcGMP or sodium butyrate, there was no effect on growth, morphology, or the relative rate of collagen synthesis, thus indicating a specificity for the dibutyryl analog of cAMP. Growth of the parental line, BALB 3T3, was inhibited by 0.5 mM dbcAMP, but the relative rate of collagen synthesis was unchanged. These results suggest that although growth, morphology, and collagen synthesis are altered in transformed cells so that they more closely resemble these characteristics in the parental line, each property may be regulated independently.

6825 INITIATION OF ONCOGENIC TRANSFORMATION OF MOUSE LYMPHOCYTES *IN VITRO* BY ABELSON LEUKEMIA VIRUS. *(E.)* Sklar, M. D. (Natl. Inst. Allergy Infect. Dis., Bethesda, Md.), B. J. White and W. P. Rowe. *Proc Natl Acad Sci USA* 71(10):4077-4081, 1974.

The possibility that C-type RNA viruses cause the oncogenic transformation of lymphoid cells *in vitro* was investigated. Splenocytes from female BALB/c mice were infected with Abelson leukemia virus at the time of plating or after 24 or 48 hr of culture. At 3-6 days after infection, transformed culture cells were injected i.p. into BALB/c or CALF1 mice (one experiment in BALB/c mice, four experiments in CALF1 mice; 2-7 mice/experiment). Four out of seven injected BALB/c mice and ten out of 12 injected CALF1 mice developed tumors. 13 of the 14 tumors were lymphomas, similar to those previously reported in BALB/c mice infected with Abelson virus. One tumor was a plasmacytoma. Mice receiving uninfected control cells did not develop tumors during 183 to 200 days of observation. Tumors arising in the initially injected CALF1 mice were transplanted again in CALF1 mice. Developing tumors in these mice were karyotyped with *Rb(5;19)1Wh* translocation in the chromosomes of metaphase cells. Except for one secondary tumor with CALF1 karyotype all tumors of secondary recipients showed no translocation (normal BALB/c karyotype). The experiments show that the secondary tumors derive from cells infected *in vitro* with Abelson leukemia virus.

6826 THE RESULTS OF CELL-CELL COLLISIONS IN THE CULTURES OF NORMAL AND TRANSFORMED FIBROBLASTS. *(Rus.)* Vasiliev, Y. M. (Acad. Med. Sci. U.S.S.R., Moscow), I. M. Gelfand, V. I. Guelstein, O. Y. Ivanova, S. G. Komm and L. B. Margolis. *Tsitologiia* 16(6):752-756, 1974.

In order to test the hypothesis that malignant cells lose the capacity for contact inhibition, cell-cell collisions were studied with the aid of time-lapse microcinematography in primary cultures of normal mouse embryo fibroblast-like cells and cultures of transformed mouse cells of two types: primary fibroblast-like cells transformed by the Moloney mouse sarcoma virus and neoplastic fibroblasts of the CIM strain. Collisions of normal fibroblast-like cells and CIM cells in mixed cultures were also analyzed. Classification of the results of collisions was based on the observation of movements of the active cell edge during the first hour after the moment when this edge had contacted another cell. All types of cell-cell collisions studied showed the same degree of contact inhibition. Thus, the altered morphology of the cultures of transformed cells cannot be explained by the loss of contact inhibition.

6827 INDUCTION OF GUINEA PIG LEUKEMIA-LIKE VIRUS FROM CULTURED GUINEA PIG CELLS. *(E.)*

Rhim, J. S. (Microbiol. Assoc., Inc., Bethesda, Md.), K. D. Wuu, H. S. Ro, M. L. Vernon and R. J. Heubner. *Proc Soc Exp Biol Med* 147(1):323-330, 1974.

The chemical induction of particles resembling guinea pig leukemia virus from normal guinea pig cells and guinea pig nonproducer (NP) cells is described. One-day-old cultures of normal or NP cells were exposed for one day to various chemicals over a range of doses. The *in vitro* COMUL test, RNA-directed DNA polymerase assay, (^{3}H)uridine-incorporating test, and electron microscopic examination were used to detect the replication of virus in these cultures. Particles resembling guinea pig leukemia virus were activated from both normal and NP cells by treatment with 5-bromodeoxyuridine (BrdU); maximum induction was usually obtained 6-8 days after exposure of the cells to 25-50 μg BrdU/ml for one day. The activated particles were approximately 100 nm in the mature form, had a density of 1.16-1.17 g/ml in sucrose density gradients, and contained RNA-dependent DNA polymerase activity. The polymerase activity was reinducible and was always detectable in the BrdU-containing medium of the cultures in prolonged cultivation. The thymidine analogs (BrdU and 5-iododeoxyuridine) were by far the most efficient inducers of the guinea pig leukemia-like virus in these cells, although 5-fluorouracil, 2-amino-6-mercaptopurine, and 3-methylcholanthrene were slightly to moderately effective. The results indicate that these virus particles are probably truly endogenous virus, and that the particles seen in leukemic cells probably result from the expression of endogenous viral DNA.

6828 DEMONSTRATION OF HELPER ACTIVITY WITH HUMAN LEUKEMIC TISSUE EXTRACTS. *(E.)*

Steeves, R. A. (Roswell Park Mem. Inst., Buffalo, N.Y.), A. Fjelde and E. A. Mirand. *Bibl Haematol* (39):357-361, 1973.

Murine leukemia virus (MuLV) increased the focus-forming efficiency of Friend spleen focus-forming virus (SFFV) under conditions in which SFFV's natural helper virus, a lymphatic leukemia-inducing virus (LLV) was inhibited. Requirements for MuLV helper activity were an N-tropic Friend virus complex and a B-type host. Subsequently, extracts of human leukemic tissues were tested for their ability to increase the spleen focus-forming activity of Friend virus complex in BALB/c mice. Helper activity was calculated by multiplying the differences (Δ foci) between focus formation (FF) with and without the addition of human extract by the dilution factor of SFFV to give a value in ΔFFU/ml; only values > 1500 ΔFFU/ml were considered positive. Helper activity was present in the cultured spleens of 20 of 31 patients with chronic hematopoietic neoplasms, but was not detected in any of 14 normal splenic or 7 fetal cultures. The disease most likely to show helper activity appeared to be chronic myelogenous leukemia (5/5 positive cultures). While a viral mechanism for the helper effect was not conclusively demonstrated, the helping agent in the human leukemic extracts was filterable, sedimentable, and was demonstrable only under conditions in which the helper virus indigenous to the Friend virus complex was inhibited.

6829 POLYRIBOADENYLATE SEQUENCES AT THE 3'-TERMINI OF RIBONUCLEIC ACID OBTAINED FROM MAMMALIAN LEUKEMIA AND SARCOMA VIRUSES. *(E.)*

Phillips, L. A. (Natl. Cancer Inst., Bethesda, Md.), J. J. Park and V. W. Hollis, Jr. *Proc Natl Acad Sci* 71(11):4366-4370, 1974.

Enzymatic analysis of 3T3 cells infected with a murine leukemia virus isolate (MLV-IC from Moloney leukemia-sarcoma virus complex and sarcoma + helper + mycoplasma negative cells) was used to find the location of the RNA polyadenylate sequences. The virus was labeled with (2,8-^{3}H) adenosine (25-50 Ci/ml) from 3-6 hr and labeled RNA was extracted. The RNA subunits were separated by rate zonal sedimentation and then fractionated. Binding to millipore filters, binding to poly(U) GFC filters and RNase A and RNase T_1 resistance techniques were used to determine if the RNA contained poly(A) sequences. Exoribonuclease from Ehrlich ascites tumor cells and polynucleotide phosphorylase (PNPase) from *Micrococcus luteus* were used to determine if the poly(A) sequences were located at the 3'-OH termini. Poly(A) sequences were separated by polyacrylamide gel electrophoresis. The resistance studies showed that poly(A) sequences comprised 6-9% and 10% of the labeled MLV-IC and S+H+ (HTG-1) viral RNA resp. Resistance was due only to the poly(A) sequences. Poly(U) filter binding indicated that 53 ± 10% of the subunit RNA's contained poly(A) sequences. Poly(A) sequences (97%) were hydrolyzed from 3'-OH termini by the exoribonuclease. Sequences of 64S MLV-IC viral RNA (68%) were phosphorolyzed. Since 58% of the sequences of 56S+H+(HTG-1) were phosphorolyzed after five min, this indicated that they were located at the 3' termini. The molecular weight of the poly(A) sequences was estimated by electrophoresis at 1×10^5. It is suggested that the poly(A) sequences may be involved in the synthesis of viral DNA by reverse transcriptase.

6830 PROPERTIES OF MOUSE LEUKEMIA VIRUSES. VIII. THE MAJOR VIRAL GLYCOPROTEIN OF FRIEND LEUKEMIA VIRUS. SEROIMMUNOLOGICAL INTERFERING AND HEMAGGLUTINATING CAPACITIES. *(E.)*

Hunsman, G. (Max-Planck Inst. Virus Res., West Germany), V. Moenning, L. Pister, E. Seifert and W. Schafer. *Virology* 62(2):307-318, 1974.

The purified major glycoprotein (GF_{71}) of Friend leukemia virus (FLV) revealed type-specific as well as species-specific and interspecies antigenicities in various types of seroimmunologic tests. That the radioactivity of labeled FLV-GP_{71} was virtually completely precipitated by its antiserum (reacting with type, species, and interspecies determinants) as well as with serum SDS-degraded Rauscher leukemia virus (reacting mainly with species determinants) and feline leukemia virus serum (reacting with interspecies determinants) indicates that the different antigenicities reside in a single molecule. FLV-GP_{71} was also able to induce the production of neutralizing antibodies, to interfere with murine leukemia viruses from two different serotypes (Gross leukemia and FLV), and to hemagglutinate. Demonstration of hemagglutination became possible after reconstitution of GP_{71} to multivalent complexes by suitable species-specific antibody (indirect hemagglutination). It is suggested that GP_{71} plays a central role in the development of immunity against infection with C-type viruses.

6831 PREGNANCY DEPENDENCE OF MAMMARY TUMORS IN STRAIN DDD MICE.

(E.) Matsuzawa, A. (Inst. Med. Sch., Univ. Tokyo, Japan), T. Yamamoto and K. Suzuki. *J Natl Cancer Inst* 52(2):449-454, 1974.

Twenty-two mammary tumors from retired breeder DDD mice aged approximately 12 months were transplanted into virgin mice, half of which were force-bred so that the tumors could be examined for pregnancy-dependence. The influence of pregnancy on tumor growth was determined by comparing graft-take rate, latent period, final tumor weight and growth curves between virgin and breeding groups. Four tumors were classified as completely pregnancy-dependent (CPD), two as moderately pregnancy-dependent (MPD), three as slightly pregnancy-dependent (SPD) and 13 as pregnancy-independent (PID). The CPD tumors did not grow in virgins but grew rapidly in pregnant breeders and regressed after parturition, reaching ascending peaks in successive pregnancies. Morphologically, all CPD tumors were type A; composed of glandular spaces of various sizes surrounded by a single layer of small, cuboidal epithelial cells and filled with a milky secretion. One MPD tumor was a pale-cell carcinoma. Two of the SPD tumors were type A + B and the third, type P. PID tumors consisted of three type A + B tumors without secretory activity, five type P, three pale-cell carcinomas and two mixed tumors. These findings suggest that the mammary tumor virus of strain DDD may belong to a plaque-inducing mammary tumor virus.

6832 A LOCUS AFFECTING CIRCULATING INTERFERON LEVELS INDUCED BY MOUSE MAMMARY TUMOR VIRUS.

(E.) De Mayer, E. (Inst. Radium, Univ. Paris-Sud, Orsay, France), J. De Maeyer-Guignard, W. T. Hall and D. W. Bailey. *J Gen Virol* 23(2):209-211, 1974.

A possible genetic influence on the production of virus-induced circulating interferon was investigated. C57BL/6, BALB/6, F_1 hybrid, F_2 hybrid, and backcross mice were inoculated i.v. with C3H/He skim milk containing B-particles or with B-particle negative C57BL milk. The serum interferon levels were assayed at various intervals thereafter. The C57BL lines produced about three times as much interferon as did the BALB/c mice. The F_1 hybrids were all low producers, while some of the F_2 hybrids and backcrosses were low producers and some were high producers. Mendelian analysis, combined with the use of recombinant-inbred strains, indicated the existence of a locus with a quantitative effect on the circulating interferon levels induced by the mouse mammary tumor virus. This locus segregates independently from the locus which affects NDV-induced interferon levels (*If-1*) and has been tentatively designated *If-2*.

6833 SURFACE STRUCTURE OF MOUSE MAMMARY TUMOR VIRUS.

(E.) Sarkar, N. H. (Inst. Med. Res., Camden, N.J.) and D. H. Moore. *Virology* 61 (1):38-55, 1974.

The envelope of the mouse mammary tumor virus (MuMTV) was studied to determine whether the surface projections have a structural symmetry similar to that of myxo and para-myxoviruses. Viral particles were purified from mouse milk and subjected to either negative staining, thin sectioning, or freeze drying, and electron microscopy. The negatively stained, and the thin sectioned specimens showed surface projections, each connected to the virion by a thin stalk and with a distal knob. The projections were arranged mostly with a sixfold symmetry, although some were arranged in fivefold symmetry. The interprojection distance was estimated to be 73.7Å. A reticular network was revealed on the viral membrane, consisting of hexagonal and pentagonal units, and to which the projections were apparantly attached at the corners. Freeze drying and shadowing showed a regular array of pits on the virion surface, also in sixfold and fivefold symmetry. Interpit distance was 218±43 Å. The pits are believed to be a unique morphological feature of the MuMTV envelope. Some hypotheses are given to explain the origin and arrangement of the pits. The results suggest that projections of enveloped viruses are more ordered structures than commonly thought. The hexagonal and pentagonal symmetries indicate that the virion is quasi-icosohedral in form, similar to a geodesic dome.

6834 PURIFICATION AND SUBUNIT STRUCTURE OF DEOXYRIBONUCLEIC ACID-DEPENDENT RIBONUCLEIC ACID POLYMERASE I FROM THE MOUSE MYELOMA, MOPC 315.

(E.) Schwartz, L. B. (Dep. Biol. Chem., Washington U., St. Louis, Mo.) and R. G. Roeder. *J Biol Chem* 249(18):5898-5906, 1974.

DNA-dependent RNA polymerase was purified from solubilized mouse myeloma (MOPC 315) nucleoli and subjected to chromatography on CM-Sephadex, DEAE-Sephadex, and phosphocellulose ion exchange resins and to sedimentation in sucrose gradients. The resultant chromatographically homogeneous enzyme has an enzyme molecular weight of 400,000-500,000. Analysis of the purified enzyme by polyacrylamide gel electrophoresis under nondenaturing conditions revealed two protein bands which were subsequently analyzed for enzyme activity. The slower band contained significant enzyme activity, while the faster band appeared inactive. The slower band contained six subunits:

195,000 (Ia), 117,000 (Ib), 60,500 (Ic), 50,500 (Id), 27,000 (Ie), and 16,500 (If) daltons. The molar ratios of all enzyme subunits were unity, indicating a molecular weight of 466,500 for the active RNA polymerase I. The faster band was identical with the slower band except that subunit Ic was absent. MOPC 315 cells have approximately 15 x 10^4 enzyme molecules/cell, while the concentrations in other cell types are estimated to be as much as 25-fold lower. However, the specific activities and subunit structures of the enzymes purified from plasmacytoma cells and normal tissues appear similar. Thus, fluctuations in the cellular levels of RNA polymerase I activity could be the result of changes in the cellular concentration and not alterations in the catalytic efficiency of RNA polymerase I molecules. The enzyme might directly determine the rates of rRNA synthesis; nondividing cells would therefore have low enzyme levels, while dividing cells would have higher levels since a net accumulation of rRNA is required.

6835 INHIBITION OF DNA AND RNA POLYMERASES OF ONCORNAVIRUSES AND OF BACTERIA BY N-FORMYL-GLYCYL AND N-FORMYL-ALANYL DERIVATIVES OF DISTAMYCIN A.

(E.) Chandra, P. (Gustav-Embden Cent. Biol. Chem., Frankfurt, Germany), S.Hausmann and A. Metz. *Pharmacol Res Commun* 6(4):311-318, 1974.

The activity of two new derivatives of Distamycin A are described, in which the formyl group of the side chain was replaced by N-formyl-glycine or N-formyl-alanine. The effect of Distamycin A and its amino acid derivatives on the template activity of calf thymus DNA in RNA-polymerase (isolated from *E. coli* B cells) was determined by adding equimolar concentrations of the antibotics (8 x 10^{-5} M) to the incubation mixture, and starting the reaction immediately with the enzyme. Under these conditions, Distamycin A inhibited approximately 70% of the incorporation of ^{3}H-AMP into RNA. The N-formyl glycine and N-formyl alanine derivatives at the same molar concentrations were much less effective in inhibiting the template activity of DNA. The effect of Distamycin A and its two derivatives on the DNA polymerase of Friend leukemia virions was also studied. Distamycin A at 2 x 10^{-4} M inhibited about 75% of the endogenic reaction; the glycine derivative at the same molar concentration produced a 60% inhibition of the reaction and the alanine derivative also showed a poor activity. It is concluded that the formyl group must be linked to the 4-amino-methyl pyrrole-2-carboxylic acid residue to achieve its full activity in biological and biochemical systems.

6836 *IN VITRO* POLYOMA DNA SYNTHESIS: DISCONTINUOUS CHAIN GROWTH.

(E.) Francke, B. (Salk Inst. Biol. Stud., San Diego, Calif.) and T. Hunter. *J Mol Biol* 83(1):99-121, 1974.

The formation, properties, and function of the DNA fragments that occur during polyoma DNA replication were studied using an *in vitro* system for the synthesis of polyoma DNA from polyoma-infected mouse BALB/3T3 cells. The results indicated that short pulses of ^{32}P-labeled deoxynucleoside triphosphates were incorporated into the viral replicative intermediates. These intermediates yielded, on denaturation, a heterogeneous long class of DNA of up to 16 S and a discrete short class of 5 S pieces. The kinetic analysis of the appearance of the radioactivity in the long and the short chains demonstrated that equal amounts were incorporated at the beginning into these two classes. However, the rate of incorporation into the short chains plateaued, while the rate of incorporation into the long chains doubled. This suggested that the synthesis of the long chains occurred as a separate event. Polyoma synthesis may proceed by a mechanism in which nascent DNA is elongated continuously on one side; on the other side, DNA is synthesized discontinuously as 5 S fragments.

6837 INTERCONVERSION OF MOLECULAR SIZE OF THE DNA POLYMERASE FROM RAUSCHER LEUKEMIA VIRUS.

(E.) Nakajima, K. (Aichi Cancer Cent. Res. Inst., Nagoya, Japan), K. Ono and Y. Ito. *Intervirology* 3(5/6):332-341, 1974.

A molecular profile of the DNA polymerase from Rauscher leukemia virus which indicates the conversion between the high and low molecular weight DNA polymerases in the absence or presence of salt was presented. DNA polymerase was purified about 150-fold from Rauscher leukemia virus by repeated chromatography on DEAE-Sephadex A-50 with a gradient elution of 0-1.0 M KCl. The DNA polymerase purified was an aggregate enzyme which could exist in the form of 480,000 daltons molecular weight. The polymerase was converted to 135,000- and 70,000-dalton molecules when the enzyme was exposed to salt (0.5 M KCl). A species of 70,000 daltons molecular weight was the minimum size of the enzyme's active form. Removal of the salt by gel filtration permitted reversibility to the high molecular weight molecule. When measured by electrophoresis in a gel of 5% polyacrylamide containing 0.1% sodium dodecyl sulfate, the subunit constituent of the purified Rauscher leukemia virus DNA polymerase showed a polypeptide with a molecular weight of 26,000 daltons. The high molecular weight enzyme obtained in these experiments showed both RNA- and DNA-directed DNA polymerase activities, especially the template preference for poly rA:dT_{12-18} over poly dA:dT_{12-18}. The biological implications of the molecular interconversion between Rauscher leukemia virus DNA polymerases are not yet understood.

6838 CHARACTERIZATION OF THE END GROUPS OF RNA OF ROUS SARCOMA VIRUS.

(E.) Keith, J. (Dep. Mol. Biol. Virus Lab., Berkeley, Calif.), M. Gleason and H. Fraenkel-Conrat. *Proc Natl Acad Sci* 71(11): 4371-4375, 1974.

Chick embryo fibroblasts infected with Rous sarcoma virus (RSV) were used to study the end groups of the 30-35S subunits. For 3' end group analysis, the virus was labeled with a mixture of four ^{3}H-labeled nucleosides (adenosine, guanine, cytidine and uridine). The labeled virus was collected at four-hr intervals for one to two days, and the viral RNA was extracted. The RNA, together with carrier RNA of tobacco mosaic virus, was hydrolyzed and subjected to two-phase electrophoresis, followed by chromatography and elution of the spots. The results showed that only adenosine occurred in stoichiometrically significant and quantitatively reproducible amounts, corresponding to the end group of an RNA of about 10,000 nucleotides and a molecular weight of 3.2 x 10^6. The viral RNA was also fractionated on oligo(dT)-cellulose columns to yield a fraction bound at high salt concentrations and a fraction that was not bound. Terminal nucleoside analysis of the unbound RNA indicated that adenosine was also the predominant end group in this fraction. The bound fraction, after low-salt elution, was degraded with pancreatic plus T1 RNase and refractionated on oligo(dT)-cellulose to yield again a bound fraction (viral poly(A)) and an unbound fraction (the bulk of the degradation products). Results of analysis of the poly(A) fraction for 3' end groups indicated that it contained one adenosine per 130 adenylic acid molecules, in amount equivalent to a molecular weight for the 30-35S RNA of 2.7 x 10^6. For 5' end group analysis, the RSV was labeled with ^{32}P and, in separate tests, collected either at four-hr intervals for two days or for 3-min intervals for four hr. In these tests, the viral RNA was extracted, hydrolyzed, neutralized, concentrated by lyophilization, and subjected to two-dimensional thin-layer chromatography. The results showed that the 5' terminus was not di- or triphosphorylated, either when the virus was collected at four-hr intervals or within 3 min of its release from the infected cells. The lack of terminal 5'-phosphates in virus collected at the 3-min period indicated that the lack at the four-hr period was not due to an artifact imposed by the longer period. The findings confirm those of previous investigators in showing that RSV RNA terminated, like RNA of TMV and other plant viruses, in 5'-unphosphorylated adenosine. The possibility is suggested that both types of RNA are dephosphorylated enzymatically, or partly degraded from the 5' end, as soon as they are made.

6839 POLY(RIBOADENYLIC ACID) AND ADJACENT NUCLEOTIDES IN ROUS SARCOMA VIRUS RNA.

(E.) Quade, K. (Duke Univ. Med. Cent., Durham, N. C.), R. E. Smith and J. L. Nichols. *Virology* 62(1): 60-70, 1974.

Poly(riboadenylic acid) segments were released from Rous sarcoma virus 70 S RNA by the action of T_1 or pancreatic ribonuclease in high ionic strength buffer, and purified by affinity chromatography on columns of poly(ribouridylic acid)-cellulose. By oligonucleotide mapping, the poly(A) was shown to be located at the 3'-OH end of the RNA molecule and to consist entirely of approximately 200 adenylate residues. Identification of the nucleotides adjacent to the poly(A) segments was accomplished by digestion of the purified T_1 ribonuclease-released fragments with U_2 ribonuclease. C-Ap was the predominent component released by U_2 ribonuclease, indicating that most (80%) of the 35 S RNA chains terminate with the sequence 5'-(G)C-poly(A)-3'. Approximately 9% of the chains are terminated by the following sequences: 5'-(G)U-U-U-U-poly(A)-3' and 5'-(G-)(U_2C)-poly(A)-3'. The reason for presence of these two minor sequences adjacent to the poly(A) segment is not understood at present.

6840 VIROGENIC NONTRANSFORMED CELLS ISOLATED FOLLOWING INFECTION OF NORMAL RAT KIDNEY CELLS WITH B77 STRAIN ROUS SARCOMA VIRUS. *(E.)*

Boettiger, D. (Imperial Cancer Res. Fund Lab., London, England). *Cell* 3(1):71-76, 1974.

Evidence for infections in which a complete Rous sarcoma virus provirus was present in the infected cell in the absence of viral transformation was investigated using normal rat kidney cells infected with B77 strain Rous sarcoma virus. Cultures that contained morphologically normal cells only and that had displayed no evidence of morphological transformation 24 days after infection were selected for further investigation. Between 10^4 and 10^6 of these cells were fused with inactivated Sendai virus to 5 x 10^5 chicken embryo fibroblasts in monolayer culture. This led to the production of virus from the heterocaryons formed. Stocks of rescued virus were obtained by removing the agar from a plate used in a fusion experiment and transferring the cells. Virus was harvested 3-4 days after transfer. The rescue frequency of virus from transformed clones was at least 100-fold higher than the rescue of virus from nontransformed clones. To increase the frequency of virus rescue, B77-infected nontransformed cells were grown for 48 hr in the presence of 5-bromodeoxyuridine at 25 μg/ml before cell fusion. A marked increase in virus rescue frequency was observed in all cases. Although the Rous sarcoma virus-infected nontransformed cells were indistinguishable from normal rat kidney cells by at least three parameters of transformation (cell morphology, colony-forming in soft agar, and release of a factor from the transformed cells that combines with serum factor to yield proteolytic activity), they nevertheless contained a complete viral genome. It is suggested that the limitation of viral expression is at the level of viral RNA transcription and that this varies from infected cell to infected cell. Some infected cells become transformed while others do not. Variations in transcription due to spontaneous changes in the physiological state of the cell could lead to provirus transcription and hence to cell transformation.

6841 STRUCTURE AND MOLECULAR WEIGHT OF THE 60-70S AND THE 30-40S RNA OF THE ROUS SARCOMA VIRUS. *(E.)*

Mangel, W. F. (Imp. Cancer Res. Fund Lab., London, England), H. Delius and P. H. Duesberg. *Proc Natl Acad Sci USA* 71(11):4541-4545, 1974.

The effects of the bacteriophage T_4 gene-32 protein on the sedimentation properties of 30-40S RNA and 60-70S RNA of the Rous sarcoma virus (RSV) were assessed. Incubation of 60-70S RNA with gene-32 protein at low ionic strength and 38 C did not shift the sedimentation peak of the RNA. When 60-70S RNA was heated to obtain 30-40S RNA and incubated with gene-32 protein under the above conditions, more than 50% of the 30-40S RNA was degraded. Electron micrographs of the 30-40S RSV RNA revealed a linear molecule in the presence of gene-32 protein and a high degree of secondary structure without gene-32 protein. Electron micrographs of 60-70S RNA-gene-32 protein complexes showed several molecules held together at many different points. Length limits of this 60-70S RNA were calculated between 6.0 and 8.0 μm while those of the 30-40S RNA were calculated between 2.8 μm and 3.5 or 4.0 μm. Histograms of 60-70S RNA revealed a second peak in the length distribition (2.8 to 4.2 μm) which corresponds to the upper length distribution of 30-40S RNA. It is suggested that the 60-70S RNA is a linear molecule with two free ends or a 30-40S RNA molecule with a small molecule attached to it. Previous studies have demonstrated that small molecules obtained from heating 60-70S RNA have the same composition as 30-40S RNA and that the infectivity of RNA tumor virus is proportional to the amount of 30-40S RNA obtainable. Such findings together with the results presented are consistent with the proposal that 60-70S RNA molecules contain two identical 30-40S RNA subunits.

6842 RNA-DIRECTED DNA POLYMERASE AND THE REPLICATION OF ROUS SARCOMA VIRUS. *(E.)* Bishop, J. M. (Dep. Microbiol., Univ. California, San Francisco), A. J. Faras, G. C. Hansen, N. Jackson, W. Levinson, J. M. Taylor and H. E. Varmus. *Collect Pap Annu Symp Fundam Cancer Res* 25:229-257, 1974.

Several fundamental predictions which are implicit in the provirus theory of Temin were examined. To test these predictions, studies were made of DNA synthesis, using purified DNA polymerase from Rous sarcoma virus (RSV). The purified enzyme was found to be capable of responding to each of three different classes of templates, exemplified by RSV RNA, rA·oligo dT, and nicked-native DNA. Thus, the predicted unique properties of the enzyme *vis-à-vis* polynucleotide templates were not fulfilled. All of the single-stranded DNA synthesized by the DNA polymerase, using 70S RNA template, and half the double-stranded DNA synthesized by the DNA polymerase were complementary to nucleotide sequences in the template RNA. However, the chain length of the DNA product was inordinately small and, therefore, the bulk of the double-helical product represented a very limited portion of the total viral genome. Thus, the prediction that the entire viral genome must be transcribed into DNA also was not fulfilled. The prediction that if viral genome is replicated by transcription from virus-specific DNA, there is no need for complementary RNA template in the infected cell had supportive evidence in the failure of detection of complementary RNA. Examination of cells which contained no viral DNA prior to infection, using new techniques for measurement of virus-specific DNA, further supported the theory in so far as RNA tumor viruses are believed to replicate by way of a DNA intermediate. Virus-specific DNA was also found in uninfected cells of the natural hosts for these viruses. It is concluded that the synthetic capacities of the purified RSV DNA polymerase do not readily qualify this enzyme for its alleged biological function.

6843 ABSENCE OF RSV PRODUCTION IN ENUCLEATED RSV-TRANSFORMED VIROGENIC MAMMALIAN CELLS FUSED WITH CHICK EMBRYO FIBROBLASTS. *(E.)* Donner, L. (Inst. Exp. Biol. Genet., Czechoslovak Acad. Sci., Prague), L. Turek and J. Svoboda. *Int J Cancer* 14(5):657-661, 1974.

Cells of a Rous sarcoma virus (RSV)-transformed virogenic Chinese hamster line (RSCH) were enucleated by combining the actions of G forces and cytochalasin B; the enucleated cells were fused with Rous inhibitory factor (RIF)-free chick embryo fibroblasts (CEF) using beta-propiolactone-inactivated Sendai virus. Most of the enucleated RSCH cells were capable of relatively fast attachment and spreading in cell culture and remained viable for 10 hr after seeding. Enucleated RSCH cells showed roughly the same capacity for fusion with CEF as did nucleate RSCH cells. Unlike the nucleate RSCH cells, the enucleated RSCH cells did not produce RSV after fusion with CEF. This fact can best be explained by the absence of complete RSV RNA in the cytoplasm of RSV-transformed virogenic mammalian cells, possibly as a result of a partial block of RSV DNA transcription. The failure to rescue RSV from enucleated RSCH by fusion with CEF also suggests that the RSV DNA provirus is integrated exclusively in the nucleus of virogenic cells.

6844 EVIDENCE FOR NON-REPETITIVE SUBUNITS IN THE GENOME OF ROUS SARCOMA VIRUS. *(E.)* Taylor, J. M. (Dep. Microbiol., Univ. California, San Francisco), H. E. Varmus, A. J. Faras, W. E. Levinson and J. M. Bishop. *J Mol Biol* 84(1):217-221, 1974.

The complexity of Rous sarcoma virus (RS) RNA was determined using molecular hybridization. Labeled DNA complementary to both RS and polio virus RNA was synthesized using the RNA-directed DNA polymerase of RS. RNA complexities were determined from the kinetics of annealing of DNA to its templated RNA. The rates of annealing of RS virus RNA were also observed with the Schmidt-Ruppin strain, the Prague C strain and two strains of Rous-associated (RA) virus (RA virus-0 and RA virus-2). Relative to polio virus RNA, the complexity of RS is 9.3×10^6 daltons, a value close to its physically determined molecular weight of about 10^7. The correspondence between the complexity and molecular weight appears to rule out the possibility that the genome is composed of identical subunits. Although they are not present in the labeled DNA, large repeated sequences might exist but this is unlikely since the labeled DNA was shown to consist of relatively uniform transcripts of the RS virus RNA. An interpretation of these data might be that the 35S RNA subunits of the 70S virus genome are non-repetitive (each possesses a unique nucleotide sequence).

6845 ANALYSIS OF MINIMAL FUNCTIONS OF SIMIAN VIRUS 40. III. EVIDENCE FOR "HOST CELL REPAIR" OF ONCOGENICITY AND INFECTIVITY OF UV-IRRADIATED SIMIAN VIRUS 40.

(E.) Seemayer, N. H. (Wistar Inst., Philadelphia, Pa.) and V. Defendi. *J Virol* 13(1):36-41, 1974.

Syrian hamster kidney (CV-1) cell cultures were infected with UV-irradiated simian virus 40 (SV40) from "complete" and "defective" pools. Medium containing caffeine (1, 2, and 5 mM) or hydroxyurea (HU, 1 mM) was added to the cultures after 1 hr of virus adsorption at 37 C. The transforming capacity of SV40 in CV-1 cells was enhanced by a UV irradiation period of two min and remained high after stronger UV irradiation. However, in the presence of caffeine, the transformation frequency was greatly reduced even at the lowest dosage of UV irradiation (2500 erg/mm^2), while there was no reduction in the efficiency of transformation in the absence of caffeine. The relative enhancement observed with the two-min UV SV40 was destroyed by caffeine, but the residual transformation frequency still reached a higher value than for the 30-sec or one-min irradiated samples. Similar results were obtained with both "complete" and "defective" virus; caffeine did not alter the transformation frequency of non-irradiated virus. In the presence of caffeine, the infectivity of the UV-irradiated SV40 decreased at a faster rate than in the absence of caffeine. It was apparent that, even in the presence of caffeine, the transformation capacity of the virus was more resistant than its infectivity. The infectivity of UV-irradiated SV40 was greatly decreased by caffeine. Addition of HU to the culture medium only slightly decreased the transformation efficiency of UV-irradiated and nonirradiated SV40, indicating that the early events in the transformation process proceed under conditions of highly depressed normal DNA synthesis.

6846 SOME PROPERTIES OF A MIGRATION FACTOR FROM A TRANSFORMED CELL LINE.

(E.) Burk, R. R. (Imp. Cancer Res. Fund Lab., London, England). *Cold Spring Harbor Conf. Cell Proliferation* 1:27-35, 1974.

An inherent factor that may stimulate the overgrowth of 3T3 cells was obtained from SV29 cells (Simian virus 40-transformed BHK). Purification of the factor after its fractionation on G75 Sephadex was dependent on addition of phosphate to the starting medium. The factor has an isoelectric point of about 10.4, a molecular wt of about 40,000 daltons, is stable at pH 1-12 at 4 C for several hr, and is completely inactivated by pepsin at 10 μg/ml. The fraction stimulated 3T3 cells to incorporate tritiated thymidine not only in cells which migrate, but also in those in the monolayer of plates used for migration assays. Activity of the fraction was 5000-fold higher than that of calf serum; unlike calf serum, the migration factor produced only a small increase in phosphate transport in 3T3 cells. The association of a topological effect on thymidine labeling with migration is reminiscent of the association between density inhibition of growth and contact inhibition of movement. A migration factor might therefore explain the correlated loss of density inhibition of growth and contact inhibition of movement observed after transformation. The invasivness and metastasis of SV29 cells, as well as their transformed characteristics, might be due to an increased ability to migrate.

6847 CHARACTERIZATION OF "HEAVY" AND "LIGHT" SV40-LIKE PARTICLES FROM A PATIENT WITH PML.

(E.) Martin, M. A. (Natl. Inst. Health, Bethesda, Md.), L. D. Gelb, C. Garon, K. Takemoto, T. N. H. Lee, G. H. Sack, Jr. and D. Nathans. *Virology* 59(1):179-189, 1974.

The properties of a virus isolated from a patient with progressive multifocal leukoencephalopathy were compared to those of simian virus 40 (SV40). Primary African green monkey kidney cells were infected at a multiplicity of 50 plaque-forming units per cell and cytopathic effect was noted by 72 hr. Centrifugation in CsCl 35,000 rpm for 16 hr at 5 C resulted in a light (1.331 g/cc) and a heavy (1.337 g/cc) band. Under the above conditions SV40 banded at a density of 1.337 g/cc. The titers of the light and heavy virus particles were 4.0 x 10^8 and 3.0 x 10^5 plaque forming units/μg, resp. Fluorescent antibody tests were used to evaluate the synthesis of virion and tumor antigens by the particles and 75% of the cells infected with the heavy virions were positive. Only one cell in 10^5 infected with light virus was positive for these antigens. BALB/3T3 mouse cells were infected with heavy or light virions (1 μg virus for two hr). Three weeks after infection, cultures infected with heavy virus exhibited transformation and were positive for tumor antigens while those infected with light virus remained unchanged and were negative for tumor antigens When purified heavy and light virions were treated with 1% sodium dodecyl sulfate and then centrifuged to equilibrium in CsCl-ethidium bromide density gradients, twisted circular DNA molecules were isolated. The DNA from the heavy particles cosedimented (53S) with SV40 DNA I under alkaline conditions; the light DNA sedimented slightly more slowly (47S) than SV40 DNA I. Length measurements of pancreatic DNase broke molecules were assessed by microscopy and found to be heavy (1.50±0.04 μm), light (1.19±0.06 μm), SV40 DNA (1.52±0.05 μm). ^{32}P-labeled heavy or light DNA fragments were allowed to reassociate at a concentration of 2.04 x 10^{-4} OD/ml in 0.14 phosphate buffer. Heavy fragments reassociated with a C_0t of 1.9 x 10^{-3} while light fragments reassociated with a C_0t of 7.5 x 10^{-4}. These results suggest that the light viral DNA is only 40% as complex as the heavy viral DNA. Digestion of light viral DNA with *Hemophilus influenzae* endonuclease resulted in five fragments which did not correspond to any of the 11 fragments derived from plaque-purified virus isolated from a patient with progressive multifocal leukoencephalophy or from SV40 DNA. Infection of African green monkey kidney cells with heavy virus gave rise to both large and small plaques. One out of four small-plaque virus stocks gave rise to two bands suggesting that ight virus particles arise during the replication f heavy virions. While it is possible that light irions contaminated the one positive plaque, the roposed theory suggests a complex process involving ultistep recombination and reassortment events hich result in the appearance of light and heavy iral DNA particles much like the evolution of discrete species reported for small-plaque SV40.

848 CATTLE LEUKEMIA LINKED TO VIRUS. *(E.)* Anonymous. *Agric Res* 22(12):12-13, 1974. (12):12-13, 1974.

849 RHABDOMYOSARCOMAS INDUCED BY ROUS SARCOMA VIRUS IN GOLDEN HAMSTERS. *(E.)* Leven- ook, I. (L. A. Tarassevich State Res. Inst. Stand. ontrol Med. Bio. Prep., Moscow, USSR), O. Kolomyetz, . Polukhina-Nikolayeva and E. Tsetlin. *Folia Biol raha)* 20(4):238-243, 1974.

850 EFFECTS OF FRIEND LEUKEMIA VIRUS ON ANTIBODY-FORMATION OF MICE TO HUMAN ERYTHRO- TES. *(E.)* Ordene, K. W. (Dep. Biol., Lafayette oll., Easton, Pa.), L. T. Stableford and S. K. ajumdar. *Proc Pa Acad Sci* 48:52-54, 1974.

351 PURIFICATION OF CV VIRUS BY CsCl DENSITY GRADIENT CENTRIFUGATION AND EXTRACTION OF IRAL DNA BY MAK COLUMN CHROMATOGRAPHY. *(E.)* lvarez-Garcia, M. C. (Inst. Natl. Oncol., Ciudad niv., Madrid, Spain), Y. Valladares and Y. Alvarez. *entralbl Bakteriol* [*Orig A*] 229(3):343-351, 1974.

852 DUAL SUSCEPTIBILITY OF A 3T3 MOUSE CELL LINE TO INFECTION BY N- AND B-TROPIC MURINE EUKEMIA VIRUS: APPARENT LACK OF EXPRESSION OF THE /-1 GENE. *(E.)* Gisselbrecht, S. (Natl. Cancer nst., Bethesda, Md.), R. H. Bassin, B. I. Gerwin nd A. Rein. *Int J Cancer* 14(1):106-113, 1974.

853 VIRION AND TUMOR CELL ANTIGENS OF C-TYPE RNA TUMOR VIRUSES. *(E.)* Bauer, H. (Robert och-Inst., Berlin, Germany). *Adv Cancer Res* 20:275- 41, 1974.

854 DIFFERENCES BETWEEN MURINE LEUKEMIA VIRUS AND MURINE SARCOMA VIRUS: EFFECTS OF VIR- ON AGE AND MULTIPLICITY OF INFECTION ON VIRAL RNA. *.)* Riggin, C. H. (Dep. Microbiol. Mol. Biol., anderbilt Univ., Nashville, Tenn.), M. C. Bondurant nd W. M. Mitchell. *Intervirology* 2(4):209-221, 1973/ 974.

6855 GROWTH DEPENDENT 2-DEOXY-D-GLUCOSE UPTAKE IN TRANSFORMED AND NORMAL MOUSE CELLS. *(E.)* Martin-De Leon, P. A. (Dep. Biol., McGill Univ., Montreal, Canada) and B. B. Mukherjee. *Can J Genet Cytol* 16(4):823-830, 1974.

6856 C-TYPE PARTICLES IN CULTURED LYMPHOCYTES FROM HIGHLY LEUKEMIC CATTLE. *(E.)* Weiland, F. (Fed. Res. Inst. Anim. Virus Dis., Tubingen, West Germany), S. Ueberschar, O. C. Straub, O. R. Kaaden and B. Dietzschold. *Intervirology* 4(3):140-149, 1974.

6857 INCREASED DNA-DEPENDENT RNA POLYMERASE ACTIVITY FROM CV-1 CELLS PRODUCTIVELY INFECTED BY SV40. *(E.)* Righthand, V. F. (Wayne State Univ. Sch. Med., Detroit, Mich.) and J. C. Bagshaw. *Intervirology* 4(3):162-170, 1974.

6858 COMBINED INFECTION OF BALB/c MICE WITH MOLONEY VIRUS (MLV) AND PLASMODIA. *(E.)* Wedderburn, N. (No affiliation) and M. H. Salaman. *Ann R Coll Surg Engl* 55(6):272, 1974.

6859 ISOLATION OF AVIAN NEPHROBLASTOMA VIRUS FROM AVIAN MYELOBLASTOSIS VIRUS BY THE INFECTIOUS DNA TECHNIQUE. *(E.)* Ogura, H. (Inst. Virol., Giessen, West Germany), H. Gelderblom and H. Bauer. *Intervirology* 4(2):69-76, 1974.

6860 SEQUENCE HOMOLOGY STUDIES BETWEEN THE DOUBLE-STRANDED RNA GENOMES OF CYTOPLASTIC POLYHEDROSIS VIRUS, WOUND TUMOR VIRUS, AND REOVIRUS STRAINS 1, 2 AND 3. *(E.)* Martinson, H. G. (Dep. Biochem. Biophys., Univ. California, San Francesco) and L. J. Lewandowski. *Intervirology* 4(2):91-98, 1974.

See also:

* (Rev): 6601, 6616, 6617, 6618, 6619, 6620, 6621, 6622, 6623, 6624, 6625, 6626, 6627, 6628, 6629, 6630, 6631, 6633, 6634, 6636, 6650, 6654, 6672, 6677
* (Immun): 6863, 6875, 6876, 6880, 6881, 6894, 6895, 6904, 6905, 6938, 6939, 6953, 6966, 6967
* (Path): 6978
* (Epid-Biom): 7085

6861 ROLE OF IMMUNITY IN ONCOGENESIS. *(E.)* Prehn, R. T. (Inst. Cancer Res., Philadelphia, Pa.). *Biochem Dis* 4(Part B):685-689, 1974.

The role of immunostimulation in oncogenesis is discussed and studies on the mouse mammary tumor system are described. There is a possibility that, owing to nonimmunologic homeostatic controls, the nascent tumor, in the absence of immunologic stimulation, might not be able to grow. Immunostimulation may therefore determine whether the tumor can actually develop. Mammary tumors in the mouse grow to a size of several mm, but then usually become static for long periods of time. At this stage, it can be shown by transplantation experiments that further tumor growth is restricted by the presence of a non-immunologic mechanism in the surrounding normal mammary epithelium. Although the tumor cells are potentially immunogenic, the hyperplastic nodule does not immunize the host animal. The tumor apparently immunizes poorly, as long as it is confined within the anatomic limits of the mammary fat pad. If a portion of the particular tumor is inoculated outside the fat pad, the tumor in the fat pad is destroyed by a specific immune response. The fat pad is thus an immunologically privileged site owing to a defect in the afferent limb of the immune response mechanism. In this system, the tumor can attain considerable size while engendering little or no immune reaction. It would appear that the early mouse mammary tumor is a good candidate for immunostimulation to help it overcome the nonimmunologic growth controls found in the mammary gland.

6862 EFFECT OF TUMOR IMMUNITY ON THE DISTRIBUTION OF LABELED LYMPHOID CELLS IN TUMOR-CHALLENGED MICE. *(E.)* Gillette, R. W. (Meloy Lab., Inc., Springfield, Va.) and C. W. Boone. *Cell Immunol* 14(3):386-393, 1974.

The migration patterns of ^{51}Cr-labeled cells from various lymphoid organs of tumor-bearing BALB/c mice were compared with those of tumor-immunized donors that developed when such cells were injected (i.v.) into normal and tumor-challenged syngeneic recipients. The presence of tumor significantly altered the homing patterns of mature lymphoid cell populations (spleen and lymph node). Tumor immunization was produced by injection of E4 cells (1×10^6, s.c.) in the right flanks of normal BALB/c recipients and rechallenged in the opposite flank after tumor excision. Migration patterns of spleen, lymph node, thymus, bone marrow and Peyer's patch cells from tumor-immune and normal mice revealed significant shifts in the homing patterns of labeled spleen cells from E4 donors. Increased numbers of spleen- and bone marrow-seeking cells were found in spleen cell populations from tumor-immune donors. A highly significant depression in the numbers of labeled cells recovered from lymph nodes and bone marrows of tumor immune animals occurred when spleen cells from normal BALB/c donors were migrated in E4 tumor-bearing normal or E4-challenged, E4-immune mice. Homing of labeled spleen cells to spleen and small intestine was unaffected by immunity to the tumor; lymph node cells homed to the lymph nodes of E4-challenged E4 tumor-immune recipients in reduced percentages. Since distribution of lymphoid cells to the spleen and bone marrow seems consistently modified by presence of tumors or immunity to tumors, it is likely that alteration of the functional status of these organs may be fundamental to the host response to malignancy.

6863 IMMUNOLOGICAL RESPONSIVENESS IN RATS INFECTED WITH THE SOEHNER-DMOCHOWSKI MURINE SARCOMA VIRUS (MSV-SD). *(E.)* Evans, D. L. (Univ. Texas Syst. Cancer Cent., Houston), J. W. Barnett and L. Dmochowski. *Tex Rep Biol Med* 32(2):449-460, 1974.

Immunogenic responsiveness and tumor pathology were studied in New Zealand black rats inoculated at different ages with a carcinogenic murine sarcoma virus. Rats aged seven days infected with murine sarcoma virus (MSV-SD) showed erythroblastosis and anemia after 10-14 days; based on hemagglutinin levels, primary response of the infected rats to sheep RBC decreased by 75% compared to uninfected controls, and the secondary response to sheep RBC also decreased by 75%. If the rats were infected with MSV at 19 days of age, tumors developed as osteosarcomas instead, and responsiveness to the primary sheep RBC test was retained although secondary response was depressed during tumor development. MSV-induced tumor cell cultures grown in an agar overlay system were destroyed by sensitized lymphocytes from MSV-SD tumor-bearing rats but this effect was reversed in the presence of serum from MSV-SD tumor-bearing rats. These results are interpreted to indicate the presence in the serum of the tumor-bearing rats of humoral blocking antibody to MSV antigen.

6864 CEREBRAL RETICULUM CELL SARCOMA AFTER MULTIPLE RENAL TRANSPLANTS. *(E.)* Barnet, L. B. (Stanford Univ. Sch. Med., Calif.) and E. Schwartz. *J Neurol Neurosurg Psych* 37(8):966-970, 1974.

The occurence of a primary brain tumor in a 34-year old male who had undergone repeated kidney transplantations with immunosuppressive drug maintenance was described and correlations between the extreme conditions conducive to immunosuppression and carcinogenesis were drawn. The patient developed glomerulonephritis at 15 yr and renal failure ten yr later when he received hemodialysis; three yr after that, his first renal transplant was rejected within five weeks. Three more kidney transplants were performed over the next four yr. The final transplant lasted 18 months and the patient survived another seven months on hemodialysis. On autopsy he was found to have a reticulum cell sarcoma-microglioma that was histologically related to systemic histiocytic lymphoma. There was also parathyroid hyperplasia and disseminated granulomas in the lungs and peripancreatic lymph nodes and other pathology of the lymphoid system. The immunosuppressive and mutagenic factors of anti graft-rejection drug therapy (azathioprine, prednisone, actinomycin D, antilymphocytic globulin), repeated antigenic stimulation with the four kidney grafts, proliferation of possibly oncogenic viruses and chronic uremia may be related to cancer development. Although this patient is ypical of many kidney transplant recipients in which eticulum cell sarcoma develop, particularly in the rain, the prolonged etiology of this case, 25 months rom the last transplant to tumor symptoms just beore death, is unusual.

865 DELAYED HYPERSENSITIVITY RESPONSE TOWARD AUTOCHTHONOUS TUMOR EXTRACTS. *(E.)*

erberman, R. B. (Natl. Cancer Inst., Bethesda, Md.). *ecent Results Cancer Res* 47:140-146, 1974.

tudies of cellular immunity have primarily been one *in vitro*. Using crude extracts of various carinomas an *in vivo* analysis by delayed hypersensiivity reactions in the skin was made on patients ith various carcinogenic conditions. Membrane reparations were injected intradermally in 0.1 ml olumes; a battery of recall antigens were also ested to determine general cellular reactivity. A mm or more induration 48 hr after inoculations as considered a positive reaction. Positive acute eukemia patient skin tests correlated with clinical tate. The tests were positive in remission and egative in relapse. Recall antigens showed no varation with changes in the clinical state. Burkitt's umor patients displayed a similar reactivity-linical state correlation. The relapse incidence n this group was the same in positive and negative est patients, however, remission was significantly onger in positive patients. The membrane extract nd a Sephadex G-200 fraction of the solubilized umor extract from intestinal cancer patients gave ositive reactions. The skin-reactive antigen in hese cases appeared to be a carcinoembryonic antien different from that previously described in imilar work. Membrane extracts and two different ephadex G-200 fractions of the soluble sonicate of mor gave positive reactions in patients with alignant melanoma. A correlation between the tigen E fraction and clinical state were noted. mor-associated reactivity was only seen in breast ncer patients with breast tumor membranes. Solle fraction testing in breast cancer patients owed reactivity to fractions from breast tumor, rmal breast from a tumor patient and normal breast ssue from patients with a benign condition. The sefulness of the *in vivo* method is clearly demonrated by the data. The advantages are simplicity testing, no need for tissue culture, and a more alistic immune state model; disadvantages are lack precision in measurement, the need for time and tient cooperation and possible sensitization to e antigens.

66 CELL INTERACTIONS IN ADOPTIVE IMMUNE REJECTION OF A SYNGENEIC TUMOR. *(E.)* Howell,

B. (Natl. Cancer Inst., Bethesda, Md.), J. H. an, E. C. Esber and L. W. Law. *Int J Cancer* 14(5): 2-674, 1974.

e interactions of subpopulations of lymphocytes the expression of cell mediated immunity to tur-specific transplantation antigens of a syngeneic mor were investigated in BALB/c mice using the nn tumor neutralization test. The mice were rendered immune to the simian virus 40-induced tumor mKSA. When the immune lymphocytes were injected in contact with the mKSA cells, they prevented tumor growth in ten hosts, whereas ten hosts receiving normal lymphocytes and tumor developed growths. Immune lymphocytes from the same source injected i.p. at a different site, were completely ineffective even at twice the attacker-to-target ratio. Mice were totally resistant to a challenge dose of mKSA ascites cells four logs greater than the TD_{50} by seven days after immunization, and their spleen cells suppressed tumor development completely in the Winn test by 12 days after immunization. Immunity reflected by the ^{51}Chromium lymphocytotoxicity assay and microcytotoxicity test correlated poorly with *in vivo* resistance to tumor challenge and the Winn test. Investigations of the contribution of the immune donor lymphocytes to the reaction demonstrated that immune thymus-dependent lymphocytes were essential, but other donor cell populations were not. Proliferation of immune donor cells was not required. It is proposed that donor thymus-dependent lymphocytes do not attack the tumor directly, but must interact with recipient cells to generate an effector response.

6867 REDUCTION OF IMMUNOLOGICAL SURVEILLANCE LEVEL IN CANCER PATIENTS. *(E.)* Orita,

K. (Okayama Univ. Med. Sch., Japan), H. Miwa, K. Ogawa, K. Suzuki, K. Sakagami, E. Konaga, Y. Kokumai and S. Tanaka. *Gann Monogr Cancer Res* (16):53-62, 1974.

The relations of cancer development, especially of cancers of digestive tracts, to the immunological surveillance system are analyzed in terms of numbers and functions of thymus-dependent lymphocytes (T cells). Two reactions, the tuberculin test and 2,4-dinitrochlorobenzene (DNCB) test, were used. Out of 246 cases the positive rate in tuberculin sensitivity tests was high (69%) in 96 non-neoplastic disease cases while low (49%) in 150 malignant cases. With DNCB the positive reaction was 91% in 95 non-neoplastic cases while in 147 malignant neoplasm cases it was only 46%. *In vitro* blast formation by ^{3}H-thymidine uptake and morphological cell change was observed through peripheral blood lymphocytes cultured with phytohemagglutinin (PHA). Blast formation rate for 102 non-neoplastic cases was 61.7% (normal rate:65.1%), while 252 malignant neoplasm cases had about 1/2 that rate (36.7%). The average rates for gastric cancer, colon rectal cancer, breast cancer and others were 36.2, 37.6, 37.5, and 36.8 resp. In comparing blast formation to tuberculin reaction, 21% non-neoplastic responded negatively to tuberculin and 39% in the terminal stage responded positively--which does not suggest a marked correlation. In comparing blast formation to DNCB reaction the non-neoplastic cases reacted positively to DNCB while in the group of non-curative operation and palliative operation cases 90% showed blast formation rate less than 40% and 70% responded negatively to DNCB, indicating a fairly marked correlation. In investigating the mutual relationship between the incidence at T and B cells (subpopulations of animal systems lymphocytes) in peripheral blood of gastric cancer and the advance of cancer, a decreasing tendency is observed in the percentage of T cells only in advanced cancer. With the blast formation rate and DNCB tests it is possible to determine before operation whether or not the curative surgical operation is indicated.

6868 CELLULAR IMMUNITY AGAINST TUMOR-ASSOCIATED ANTIGENS IN HUMANS: LYMPHOCYTE STIMULATION AND SKIN REACTION. *(E.)* Vanky, F. (Dep. Tumor Biol., Karolinska Inst., Stockholm, Sweden), E. Klein, J. Stjernsward and U. Nilsonne. *Int J Cancer* 14(2): 277-288, 1974.

Eighteen tumor bearing patients (14 sarcomas, one Wilm's tumor, two astrocytomas and one melanoma) were tested for cell mediated reactivity against their biopsied tumors using both intact cells and KCl extracts in efforts to correlate the *in vitro* results to the reactivity of the patients *in vivo.* Lymphocytes from ten patients reacted with increased ^{3}H-incorporation when incubated with the biopsy cells. All except one reacted to corresponding KCl extracts also. Eight tumors failed to be stimulated either by cells or by KCl extracts. Seven of these were tested with different doses around the usual optimal stimulating dose (between 100 and 200 μg/ml protein concentration of the extract). One out of 18 nonmalignant tissue cell suspensions and 1/10 non-malignant KCl extracts were positive. All non-malignant tissue cells and 5/10 of their KCl extracts were used in several doses. Extracts of ten stimulating tumors induced skin reactions after intradermal injection. Three of seven extracts from ATS-negative tumors and three of 12 KCl extracts of non-malignant tissues, likewise negative *in vitro,* evoked skin reactions. The results show concordance between the ATS of cells and their KCl extracts and also between the *in vitro* and *in vivo* reactions towards the tumor KCl extracts. The few positive reactions with the non-malignant material were not consistent. The results strengthen the validity of the lymphocyte stimulation test as an assay for tumor specific reaction.

6869 IMMUNOLOGIC REACTIONS IN PATIENTS WITH NEUROBLASTOMA DURING THE COURSE OF THEIR DISEASE. *(E.)* Helson, L. (No affiliation). *Maandschr Kindergeneeskd* 42(9/10):418-422, 1974.

In evaluating specific tumor-directed cell-mediated immunity (CMI) reactions the necessity of determining whether or not CMI impairment is generalized has been documented. Fifty children with neuroblastoma were tested for their ability to develop skin sensitization with dinitrochlorobenzene (DNCB). The results were analyzed with respect to clinical state, age at onset, survival, peripheral lymphocyte counts and lymphocyte-mediated cytotoxicity. All patients were sensitized after surgery and in most cases following or concomitant with chemotherapy or radiation. The DNCB sensitizing dosage was 2000 μg; challenge followed 14 days later using 100 μg and 25 μg doses applied to the forearm. Induration and swelling at the challenge sites was considered a positive reaction. Ninety percent of the patients with localized neuroblastoma or who were free from clinically evident disease were DNCB positive; all were under two years old at diagnosis. In contrast, only 27% of the patients with disseminated neuroblastoma were DNCB reactive; these included 11 children under two years. Lymphocyte counts were lower in disseminated neuroblastoma patients, as were survival rates. The results indicate the value of CMI in estimating prognosis in children with localized neuroblastoma, or in children under two years of age at diagnosis regardless of stage. In disseminated disease cases the negative DNCB reaction is confirmation of poor prognosis while the positive reaction remains variable.

6870 CANCER IN CHILDREN WITH PRIMARY IMMUNODEFICIENCY DISEASES. *(E.)* Kersey, J. H. (Dep. Lab. Med. Pathol., Univ. Minnesota, Minneapolis), B. D. Spector and R. A. Good. *J Pediatr* 84(2):263-264, 1974.

Data from the Immunodeficiency-Cancer Registry indicated that 60 children under 15 yr of age with primary immunodeficiency disease died of cancer. Included were severe-combined immunodeficiency (nine cases), X-linked (Bruton's) agammaglobulinemia (four cases), Wiskott-Aldrich syndrome (14 cases), ataxia-telangiectasia (29 cases), variable (late-onset) immunodeficiency (three cases) and IgA deficiency (one case). The risk of death from malignancy in children with these immunodeficiency syndromes was determined to be 100 times that of the general population of children under 15 yr (0.8/100/yr *versus* 0.007/100/yr, resp.). Cases identified in the United States included 39 of the 60 cases of primary immunodeficiency disease and cancer. While leukemias predominate the general population (48% of the total), lymphoreticular solid tumors (reticulum cell sarcoma, lymphosarcoma, etc.) predominate in the primary immunodeficient population (67% of all cases and 69% of the United States cases). This predominance suggests that the increased risk of cancer in persons with primary immunodeficiency disorders may be largely due to intrinsic lymphoid abnormalities.

6871 CHEMICAL IMMUNOSUPPRESSION AND HUMAN CANCER. *(E.)* Penn, I. (Dep. Surg., Univ. Colorado Sch. Med., Denver). *Cancer* 34(4):1474-1480, 1974.

A significant advance in the understanding of cancer is the realization that states of immunodeficiency are associated with an increased incidence of malignancies. Malignant cells have been inadvertently transplanted with homografts obtained from donors with cancer. In 17 out of 47 such immunosuppressed hosts, cells survived, multiplied, and spread. In addition, 432 organ homograft recipients have shown a 5.6% incidence of *de novo* malignancies at some time after transplantation, an incidence 100 times greater than that of the general population of the same age. Organ transplant recipients who had pre-existing malignancies removed developed recurrences or metastases of the original neoplasms in 41% of cases. Malignancies have also been observed in nontransplant patients treated for a variety of disorders. Cancer chemotherapeutic agents have immunosuppressive side effects; patients have manifested new malignancies while their original tumors were controlled by the antineoplastic drugs. Hypothetically, immunosuppression may lead to oncogenesis by: direct oncogenic action; potentiation of environmental carcinogens; allowing for the growth of oncogenic viruses; and in patients with neoplasms, depression of cellular immunity. The danger of malignancy makes it imperative that immunosuppressive therapy only be used when strongly indicated, as in organ transplantation, or where other forms of therapy have failed to control the patient's disease. The use of the appropriate cancer chemotherapeutic agents in patients with advanced malignancies is fully justified, as the risk of development of new tumors is far out-weighed by the months or years of control of the original neoplasms. Chemotherapy, as an adjuvant to radiotherapy or surgery in the management of localized cancers, should be used with caution as it has yielded disappointing results in many series.

6872 MECHANISMS OF TUMOR-INDUCED IMMUNOLOGICAL DEFICIENCIES AND THEIR POSSIBLE SIGNIFICANCE N RELATION TO THE USE OF IMMUNOPOTENTIATORS IN TUMOR-BEARING HOSTS.

(E.) Kerbel, R. S. (R. Cancer Hosp., London, England). *Biomedicine* 20(4):253-261, 1974.

Several types of immunosuppressive effects resulting rom untreated tumor growth were described. The fact hat immunoenhancing therapy is unlikely to succeed under these circumstances unless ways can first be ound to restore the full immune response is emphaized. Five types of immunosuppression are presented: 1) antibody complexes with tumor specific antigen nd their reactivity with T cells, interfering ith cell-mediated immune response; (2) elevated coricosteroid levels which may cause depressed cortical hymus function; (3) synthesis of immunosuppressive actors by tumor cells, although the undefined nature f these factors and the possibility that it might be nother expression of (1) is considered; (4) preemption, he immunosuppressive effects of highly localized antien sources by alteration of the migration route of ymphocytes and (5) tumor-induced inhibition of the ymph node lymphocyte trapping process that appears to e necessary for normal immune response. Immunorestortion is thus considered an advantageous alternative o immunopotentiation.

873 LYMPHORETICULAR MALIGNANCY IN IMMUNODEFICIENCY WITH LYMPHOCYTOTOXIC ANTIBODY.

(E.) an, A. K. (Harvard Med. Sch., Boston, Mass.), A. ez-Giangreco and G. F. Vawter. *Cancer* 34(5):1612-19, 1974.

usual forms of immuno-deficiency were present in o patients (one girl, one boy; 11 and 14 yr, resp.) who developed malignancy in terminal stages. The girl suffered from repeated skin infections from 5-25 months of age, and later had orbital cellulitis, bilateral serous otitis media, chicken pox, measles, and monilial pneumonia. The patient was administered gamma globulin (i.m.) at monthly intervals. At nine yr, when the patient was profoundly lymphopenic, serum contained a complement-dependent cytotoxic activity against her own lymphocytes and against lymphocytes from a normal donor. The patient died at 11 yr; a tumor mass in the right lung morphologically resembled a plasmacytoma. At ten yr, the boy developed bilateral draining otitis media which was treated by bilateral mastoidectomies; recurrent respiratory infection with pneumonic consolidation developed after this. At 12 yr, a biopsy of the stimulated inguinal lymph node (seven days after i.m. injection of diphtheria and tetanus toxoid) showed poorly defined lymphoid follicles without germinal centers in the cortex and few lymphocytes in the paracortical areas. An allogenic skin graft was retained without rejection for two yr. The patient died at 14 yr after admission with fever and chills; tumor cells obtained in a biopsy of the right axillary lymph node resembled pleomorphic reticulum cells. Lymphocytotoxic antibody was detected in the sera of both patients and was associated with recurring lymphopenia in the first patient. A systematic search for lymphocytotoxic antibody in lymphoreticular malignancies associated with either lymphocytopenia or abnormal cell mediated immune reactions may be worthwhile.

6874 SERUM IMMUNOGLOBULINS IN PATIENTS WITH UTERINE NEOPLASMS WITH AND WITHOUT METASTASES.

(It.) Pezzullo, G. (San Giovanni di Dio Hosp., Frattamaggiore, Italy). *Clin Eur* 12(2):103-110, 1973.

Immunoglobulin fractions were correlated with the presence of neoplasms in 20 women patients with uterine carcinoma without detectable metastasis and in 20 with histologically diagnosed lymph node metastasis. IgG gave marked quantitative variations in all types of neoplasms studied, particularly in those of the uterine body; higher values were found in lymph node metastases. IgM gave no significant increases, although the amounts in cases with metastasis were higher than in those without. IgA gave no particular variations in the average values. Thus, some significance can be attached in cases of neoplasm of the uterine neck and corpus to quantitative variations of immunoglobulin sera, especially the fraction IgG.

6875 HOMOLOGOUS AND HETEROLOGOUS IMMUNIZATION AGAINST ROUS SARCOMA.

(E.) Sigel, M. M. (Univ. Miami, Fla.), H. T. Holden and P. Meyers. *Bibl Haematol* (39):698-705, 1973.

Although achievement of protection against tumor induction by Rous sarcoma virus (RSV) with the use of killed virus preparations or passive antibody had previously been unsuccessful, the present work confirmed findings that immunization of specific pathogen-free chickens with live RAV-1 can elicit strong resistance against tumor induction after challenge with RSV (RAV-1). Doses as low as 1-10 infectious U were effective. A correlation was observed between induction of resistance and development of neutralizing antibodies, but this did not denote a causal relationship since heterologous immunity independent of neutralizing antibodies developed at the same time. Thus, chickens immunized with live RAV-1, a subgroup A virus, also demonstrated high levels of resistance to challenge with the Schmidt-Ruppin strain of RSV (SR-RSV) which belongs to subgroup B and shares no discernable envelope antigens with RSV (RAV-1), even though these resistant chickens had no demonstrable neutralizing antibodies to SR-RSV. In a reciprocal experiment, chickens immunized with live RAV-6, a subgroup B avian leukosis virus, showed marked protection against tumor induction by the subgroup A virus, RSV (RAV-1), as well as protection against SR-RSV. The explanation is believed not to be due to the presence in the immunizing virus preparations of contaminants antigenically related to the challenge virus, but is suggested to be due rather to the production of common virus-induced surface or gs antigens in the infected cells.

6876 IgM ANTIBODIES SPECIFIC FOR EPSTEIN-BARR VIRUS IN INFECTIOUS MONONUCLEOSIS WITHOUT HETEROPHIL ANTIBODIES.

(E.) Nikoskelainen, J. (Dep. Virol., Univ. Turku, Finland), J. Leikola and E. Klemola. *Br Med J* 4(5936):72-75, 1974.

Immunoglobulin M (IgM) antibodies specific for Epstein-Barr virus (EBV) were demonstrable in all but one of 46 patients diagnosed as having infectious mononucleosis without heterophil antibodies; cytomegalovirus etiology was excluded. In all but two cases the highest titer was found in the first sample. In 21 patients IgM titers decreased significantly within a few weeks. IgG antibodies to EBV, mostly remaining at a constant level, were demonstrable in all cases. IgM antibodies to EBV were detected in only five of 300 controls. The results suggest that testing for EBV-specific IgM antibodies is of great value for the etiological diagnosis in diseases resembling infectious mononucleosis but without heterophil antibodies. Viruses other than EBV and cytomegalovirus are unlikely to be causal agents in such diseases.

6877 IgA AS A BLOCKING FACTOR IN HUMAN MALIGNANT MELANOMA.

(E.) O'Neill, P. A. (Texas Med. Cent., Houston) and M. M. Romsdahl. *Immunol Commun* 3(5):427-438, 1974.

An attempt was made to determine whether a serum immunoglobulin could be implicated in blocking the cytotoxicity of sensitized lymphocytes on human malignant melanoma cells. In these *in vitro* studies, two human melanoma cell lines, SH-1 and line LeCa, Strain 26-5, were utilized as target cells, with a human neuroblastoma line (IMR-32), a normal human lung line (WI-38), and a skin fibroblast line (SaNar) serving as control target cells. Lymphocytes from melanoma patients and normal subjects were tested for cytotoxicity toward human and control cell lines by standard microcytotoxicity assay, plating 200 cells/well, with a target cell to lymphocyte ratio of 1:750. The presence of specific immunoglobulins in sera and fractions was ascertained by the method of immunodiffusion using double diffusion gel plates and heavy chain-specific goat antihuman IgA, IgG, IgM, IgE, and IgD antisera. Data from the cytotoxicity assays indicated that lymphocytes from all 11 of the melanoma patients were capable of killing human melanoma target cells *in vitro*; however, these same lymphocytes showed no cytotoxicity toward human fibroblast or neuroblastoma cell lines. Sera from nine of the 11 melanoma patients blocked cytotoxic lymphocytes. IgA from melanoma serum was found to abrogate the cytotoxicity of sensitized lymphocytes on human melanoma target cells.

6878 ABSENCE OF IgA ANTIBODY TO HERPERVIRUS IN CERVICOVAGINAL SECRETIONS OF PATIENTS WITH CARCINOMA OF THE CERVIX.

(E.) Dent, P. B. (Dep. Pediatr., McMaster Univ., Hamilton, Canada) and J. Bienenstock. *Clin Immunol Immunopathol* 3(2):171-177, 1974.

To corroborate previous findings, the incidence of herpesvirus type II (HSV II) and absence of IgA antibody in HSV II cervicovaginal secretions of carcinoma patients were analyzed. Specimens from 57 patients and 28 healthy women are examined using antigens of HSV II and *Candida albicans,* a local pathogen. Antihuman fluorescent goat conjugates containing IgA and IgM antibodies and sheep IgG were used. Specificity of these antisera was confirmed by differential staining for *Candida*. Controls contained known quantities of positive and negative sera together with uninfected HSV II cells. Immunoglobulin quantitation, using WHO standard U/cc, revealed that neither patients nor controls had any IgA or IgM antibodies to HSV II in their secretions. Approximately one-third of both groups had IgA and IgG specific to *Candida*, but no IgM was present. Of the carcinoma patients, 44.4% had IgG as compared to 7.1% in the controls. It is significant that 27.8% of the patients had serum antibody to HSV II infected cells as compared to 3.6% of the controls. It is suggested that the secretory IgA antibody response is directed against virion-specific antigens, rather than cell-surface antigens of virus-infected cells. It is also suggested that HSV infection may occur without inciting a local IgA response and that elevated levels of IgA in patients with carcinoma of the cervix may reflect the presence of a chronic immunologic stimulation at the mucous surfaces of the genital tract.

6879 AN EVALUATION OF THE ISOTOPIC ANTIGLOBULIN AND CYTOSTATIC ASSAYS FOR DETECTING IMMUNIT AGAINST SV_{40} TUMORS.

(E.) Coggin, J. H. (Dep. Micro biol. Univ. Tennessee, Knoxville), K. R. Ambrose, E. L. Candler and W. H. Hannon. *Isr J Med Sci* 10(8): 904-912, 1974.

The isotopic antiglobulin technique (IAT) assay was tested to determine whether it could detect immunity

to simian virus 40 (SV_{40})-induced sarcomas following
immunization of hamsters to the minimal extent re-
quired to produce transplantation resistance. Immun-
ization was accomplished by injection of SV_{40} virus
r of killed SV_{40} hamster tumor cells, and transplan-
ation immunity was demonstrated by challenge 14 days
ater with viable tumor cells and observation of tumor
ncidence 30-60 days later. Immunization was also ac-
omplished by simply inducing sarcomas in hamsters
ith SV_{40}, and immunity in such animals was demon-
trated by means of an adoptive transfer test in which
acrophages taken from the tumor-bearing hamsters fol-
owing immunostimulation with i.p.-injected glycogen
ere mixed with viable tumor cells and injected into
-week-old normal male hamsters. In either case, the
AT assay (in which serum from the immunized hamster
as added to SV_{40} tumor cells, followed by ^{125}I-labeled
oat anti-hamster globulin) did not detect SV_{40} tumor
ntibody unless the animals had been hyperimmunized
y 5-10 injections with killed SV_{40} tumor cells. Cy-
ostatic tests of minimally immunized hamsters, con-
ucted by *in vivo* i.p. implantation of diffusion cham-
ers containing viable tumor cells and counting of
ell survivors, or by *in vitro* incubation of SV_{40} tu-
or cells (18 hr) with heat inactivated serum from im-
unized hamsters, were positive. These results sug-
est caution in using humoral immune assays alone to
valuate the presence of tumor-specific transplanta-
ion antigens.

380 ANTIBODIES TO FELINE ONCORNAVIRUS GROUP-SPECIFIC ANTIGENS IN FELINE SERA. *(E.)*

ohn, D. S. (Dep. Vet. Pathol., Ohio State Univ.,
olumbus) and R. G. Olsen. *Bibl Haematol* (39):744-
54, 1973.

determine whether the apparent inability of mice,
amsters and cats to produce complement-fixing (CF)
tibodies to group-specific (gs) antigens of RNA
mor viruses, as previously reported by other in-
stigators, might be due to innate immunotolerance,
rther experiments were carried out with cats to
st their response to feline gs antigens. A highly
nsitive complement-fixation inhibition test and a
rect paired radioiodine labeled antibody technique
re used. Feline sera were obtained from normal
d diseased conventional cats, from normal and
line leukemia virus (FeLV) infected or immunized
ecific pathogen free (SPF) cats, and from normal
d FeLV or FSV infected gnotobiotic cats. The re-
lts established that cats are not immunologically
fractory to feline RNA tumor virus gs antigens.
us, antibody titers increased during immunization
normal SPF cats with ether-extracted FeLV and
gnotobiotic cats following infection with feline
A viruses. The finding that these antibodies did
t fix guinea pig or feline complement accounted
r the failure of other investigators to demonstrate
em by the direct CF test. The apparent absence
at least detectable levels of gs-3 antibodies
sera of all but one of eight gnotobiotic cats
plied that these animals had not been immunized
the normal processes encountered by conventional
d SPF cats. The results did not exclude the pos-
bility of genetic vertical transmission of feline
A tumor virus. It appeared, however, that the concept of immunologic tolerance to gs antigens in cats should be reevaluated.

6881 NEUTRALIZING ANTIBODIES TO HERPESVIRUS TYPES 1 AND 2 IN CARCINOMA OF THE CERVIX, CARCINOMA *IN SITU* AND CERVICAL DYSPLASIA. *(E.)*

McDonald, A. D. (Dep. Epidemiol. Health, McGill Univ., Montreal, Canada), M. C. Williams, J. Manfreda and R. West. *Am J Epidemiol* 100(2):130-135, 1974.

Neutralizing antibodies to herpesvirus types 1 and 2 were estimated by microneutralization in 57 women with invasive carcinoma of the cervix, 50 women with carcinoma *in situ*, 39 women with cervical dysplasia, and equal numbers of matched controls. The ratio of type 2:type 1 herpesvirus neutralizing antibody was calculated and sera in which the ratio was 0.85 or greater were called type 2. Among the cases of invasive carcinoma and their controls, 35% of the cases and 21% of the controls were type 2; Hotelling's T^2 test, taking into account differences in titer as well as in ratio, gave a confidence of only 84% that the difference was not due to chance. For carcinoma *in situ*, the proportions were 40.0% and 32.0%, resp., and for cervical dysplasia they were 30.8% and 38.4%, resp. The controls, who were from gynecological clinics, had a higher prevalence of type 2 sera than women in the general population. The absence of a major difference in the prevalence of type 2 sera among Jewish compared with non-Jewish women suggests that their different susceptibility to carcinoma of the cervix is not connected with herpes-virus experience. The findings of this survey were inconclusive.

6882 *IN VITRO* LEUKOCYTE INTERFERON PRODUCTION IN PATIENTS WITH HODGKIN'S DISEASE. *(E.)*

Rassiga-Pidot, A. L. (Dartmouth-Hitchcock Med. Cent., Hanover, N. H.) and O. R. McIntyre. *Cancer Res* 34(11):2995-3002, 1974.

Leukocyte cultures from 21 consecutive, untreated, newly diagnosed Hodgkin's disease patients presenting from Sept. 1969 - June 1971 were tested for interferon production in response to Newcastle disease virus (NDV). Histological classification was by the Lukes-Butler criteria. Peripheral blood leukocytes were obtained by gravity sedimentation of heparinized blood samples from the untreated patients and healthy controls (20 males, 23 to 60 yr old; 9 females, 20-59 yr old). Four x 10^6 leukocytes were incubated at 37 C for 24 hr with NDV (C_6 strain). Interferon production was impaired only in Stage IV patients. There was no correlation between virus-induced interferon production and phytohemagglutinin-induced lymphocyte transformation and no correlation between interferon production and anergy in skin tests. Interferon production at time of diagnosis did not predict clinical course, but was observed to return to normal in patients in remission. The defect of interferon production in the Stage IV patients could result from: 1) a defect in the lymphocyte-macrophage interaction since added macrophages increased the production of interferon; 2) a decrease in the interferon-producing cell population; or 3) a qualitative defect in the interferon-producing cell population. The results suggest that lymphocyte-macrophage interaction may enhance NDV-induced interferon production.

6883 REPORT OF THE WORKSHOP ON THE CARCINOEMBRYONIC ANTIGEN (CEA): THE PRESENT POSITION AND PROPOSALS FOR FUTURE INVESTIGATION. *(E.)*

Neville, A. (Inst. Cancer Res., London, England) and D. J. R. Laurence. *Int J Cancer* 14(1):1-18, 1974.

Chemical and physical data, immunochemistry, assay systems and clinical applications are presented with regard to carcinoembryonic antigen (CEA), a glycoprotein (derived from human metastatic colorectal carcinoma) which is soluble in perchloric acid and strong salt solutions. Neither fucose nor sialic acid appear to be essential for specific activity. Work is needed to identify the antigenic determinants of CEA, which is proposed to involve N-acetyl-glucosamine. A sensitive, reproducible assay for clinical purposes is needed for long term follow-up studies. CEA levels tend to rise with tumor spread or mass, irrespective of the tumor type. High pre-operative levels were associated with a more rapid recurrence of tumors of the colon and bronchus; successful surgical removal of tumors is followed by a decline to normal levels in a matter of days. The presence of raised CEA levels in urine was specific to urothelial carcinoma once infection was excluded. The most valuable role for plasma CEA studies is in prognostic assessment, detection of recurrent or residual disease, and monitoring of therapy, especially with tumors of the bowel, lung, breast, kidney, pancreas and neuroblastoma.

6884 STUDIES ON THE STRUCTURE OF THE CARCINOEMBRYONIC ANTIGEN. I. SOME DEDUCTIONS ON THE BASIS OF CHEMICAL DEGRADATIONS. *(E.)*

Westwood, J. H. (R. Cancer Hosp., London, England), E. M. Bessell, M. A. Bukhari, P. Thomas and J. M. Walker. *Immunochemistry* 11(12):811-818, 1974.

The possibility that the protein chain of carcinoembryonic antigen (CEA) carries oligosaccharides which constitute the immunological determinants of the molecule was investigated using CEA from metastatic liver tumors from patients with colo-rectal carcinoma. Tumor homogenates were purified by column chromatography and each CEA sample was tested by polyacrylamide gel electrophoresis and radioimmunoassay. The analysis of all CEA monosaccharides except sialic acid was done using gas chromatography. Sialic acid was determined using a thiobarbituric acid method. Percent molar carbohydrate composition (average) was: fucose 21, mannose 10.8, galactose 28, 2-acetamido-2-deoxygalactose 2.6, 2-acetamido-2-deoxyglucose 34.8, sialic acid 2.81. Hydrolysis in 0.05 M NaOH, M NaB^3H_4 and column fractionation yielded carbohydrate-rich fragments with molecular weight greater than 6000, suggesting a branched arrangement of oligosaccharide moieties. Further degradation under stronger alkaline (M NaOH) reducing conditions yielded oligosaccharide fragments suggestive of an asparagine-2-acetamido-2-deoxyglucose type of linkage in this glycoprotein. Hydrolysis in 0.01 M H_2SO_4 at 90 C for 3.5 hr cleaved all the sialic acid and one third of the fucose. Alkaline hydrolysis destroyed carcinoembryonic antigen reactivity and binding interference in the radioimmune assay; acid hydrolysis or pepsin digestion greatly reduced these activities. Tritium labelling in the presence of galactose oxidase, of desialylated carcinoembryonic antigen indicated that all sialic acid was linked to penultimate galactose residues. It is concluded from the loss of biological activity after weak alkaline hydrolysis that most of the immunogenic properties of carcinoembryonic antigen reside in the protein moiety. While there is a remarkable consistency in the sugar composition of various tissue sources of the antigen, the ability of oligosaccharide fragments to bind to the equivalent antibody suggests the presence of carbohydrate antigenic groups and the possibility of developing more specific antisera.

6885 EMBRYONIC ANTIGENS AND GROWTH OF MURINE FIBROSARCOMATA. *(E.)*

Menard, S. (Natl. Inst. Study Cure Tumors, Milan, Italy), M. I. Colnaghi and G. Della Porta. *Br J Cancer* 30(6):524-531, 1974.

The relationship between the amount of embryonic antigen (EA) found on tumors induced by different agents and the sarcomata growth rate; EA specificity, and the influence of anti-embryo immunity on tumor growth were investigated. Tumors were induced in male and female mice of BALB/c, C3Hf and C57BL strains by either s.c. implantation of teflon disc, s.c. injection of 50 µg 7,12-dimethylbenz(a)anthracene (DMBA), fibroblast exposure to 3-methylcholanthrene (MCA), or spontaneous transformation. Tumor maintenance was by serial s.c. passage in syngeneic mice. ^{51}Cr-release with anti-embryo lymphocytes and the reduction of C57BL antiembryo serum activity following absorption with tumor cells, and also the reduction following absorption of faster dividing cells was used. Tumor growth was evaluated by the number of cells required for 50% tumor take and by the tumor growth rate. EA expression varied widely in the cytotoxic tests with 10/15 tests positive on 13 different fibrosarcomas (^{51}Cr-release=20% positive), with 3 spontaneous and 2/5 teflon disc-induced fibrosarcomas displaying the highest EA expression. A direct correlation between the number of cells required for 50% tumor take and growth rate was established, fewer cells for take yielded a faster growth rate. The absorbing capacity of the higher EA expression tumors for C57BL antiserum activity was found to be comparable to that of rapidly dividing testicular cells of pubescent and adult mice. Injections of tumor cells into mice immunized against embryo cells showed the take of small numbers of tumor cells to be higher than in untreated mice, or mice immunized against normal fibroblasts. The results imply that enhancement and resistance depend on the ratio between the level of immunity and amount of antigen; the high antigen-antibody ratio stimulated tumor growth where an increased amount of antigen failed to provide protection.

6886 BINDING OF CARCINOEMBRYONIC ANTIGEN (CEA) TO CONCANAVALIN A-SEPHAROSE: STORAGE OF HIGH-SPECIFIC-ACTIVITY ^{125}I-CEA. *(E.)* David, G. S. (Dep. Exp. Pathol., Scripps Clin. Res. Found., La Jolla, Calif.) and R. A. Reisfeld. *J Natl Cancer Inst* 53(4):1005-1010, 1974.

Binding of CEA to Sepharose-4B-conjugated concanavalin A (Con A-Sepharose) was used for long-term storage of high specific activity ^{125}I-CEA for use in radioimmunoassay. Antigenically inactive degradation products of CEA were removed by washing with buffer (pH 7 phosphate-buffered saline containing 0.1% bovine serum albumin), leaving the antigenically active CEA strongly bound to the beads. Elution was accomplished with 0.1M α-methyl-D-mannoside, which did not interfere with the antigen-antibody reaction in subsequent assays. Adsorption to and immediate elution from Con A-Sepharose slightly increased the binding of both CEA(a) and CEA(b) to specific antibody. When stored in the absence of Con A-Sepharose, the antibody-binding capacity of radiolabeled material decreased rapidly, but that eluted from Con A-Sepharose was even greater than the starting material. For example, the binding capacity after 72 days adsorption was 92% compared to 80% on day 1 and 49% after 72 days' storage without Con A-Sepharose. Sodium dodecyl sulfate disc electrophoresis indicated considerably greater homogeneity of ^{125}I-CEA after 72 days on Con A-Sepharose than when stored unadsorbed. The use of lecithin-Sepharose conjugates for the storage of other radioiodinated glycoprotein antigens for similar purposes and the possibility of taking advantage of the binding properties of Con A-Sepharose for the isolation of CEA from tumor extracts is suggested.

6887 RECENTLY DEFINED ONCO-FETAL ANTIGENS AND THEIR POTENTIAL APPLICATION IN CLINICAL MEDICINE. *(E.)* Klavins, J. V. (Long Island Jew.-Hillside Med. Cent., Jamaica, N.Y.), M. Weiss, R. Mesa-Tejada, J. A. Fierer and J. I. Berkman. *Ann Clin Lab Sci* 4(3):139-144, 1974.

Four hypothetical models of protein synthesis over the entire life span of an organism are presented depicting the possible relationships of embryonic and adult proteins as part of a search for a universally present embryonic component in malignant neoplasms. The probability of finding a specific universal embryonic antigen in human malignant neoplasms was suggested by experimental evidence from various mouse cancers. An aqueous extract from a 6-7 week old intact human fetus was used as the antigen. Ouchteriony double diffusion technique was applied to determine the reactivity. Rabbit immune serum (against the human fetal embryonic antigen) reacted with malignant neoplasms derived from all three germ layers. Thirty-one carcinomas were examined. A wide variety of non-malignant tissues were also examined and gave negative results. It is not known yet whether the immune serum contains a universal antibody against some embryonic and malignant tissue components or if there is a wide range of different antibodies which react correspondingly with a wide range of human neoplasms. It is suggested that the technique of applying rabbit immune sera, raised by fetal tissues, in diagnosis of human malignant neoplasms may be useful. (12 references)

6888 CARCINOFETAL HUMAN ISOFERRITINS IN PLACENTA AND HELA CELLS. *(E.)* Drysdale, J. W. (Tufts Univ. Sch. Med., Boston, Mass.) and R. M. Singer. *Cancer Res* 34(12):3352-3354, 1974.

Ferritins from placenta, normal liver and HeLa cells were studied in order to determine if isoferritins isolated from human tumors represent carcinofetal antigens previously isolated from hepatomas. Isoferritins from placenta, HeLa cells, and cirrhotic liver obtained from a patient with secondary iron overload were separated using polyacrylamide gel electrophoresis. In addition, some of the separated isoferritins underwent immunofixation with antibodies prepared against normal liver ferritin. The cirrhotic liver sample was resolved into six isoferritins (isoelectric points between pH 5.2-5.7), similar in pattern to normal liver isoferritins. Two isoferritins (pI's = 4.9 and 5.0) were obtained from HeLa cells; these were not detected in liver. The placenta was found to contain the isoferritins common to the liver, as well as two more that banded in the same positions as those found in HeLa cells. These were designated as carcinofetal isoferritins. The carcinofetal isoferritins were precipitated by antibodies prepared against normal liver ferritin. The finding that HeLa ferritin does not contain any of the components found in the normal liver favors the hypothesis that the two isoferritins common to placenta and HeLa cells may reflect the presence of mixed cell populations in fetal and tumor tissues.

6889 BLOOD GROUP ISOANTIGENS IN ANGIOBLASTIC MENINGIOMAS AND HEMANGIOBLASTOMAS OF THE CENTRAL NERVOUS SYSTEM. *(E.)* Jellinger, K. (Inst. Neurol., U. Vienna, Austria) and H. Denk. *Virchows Arch A Pathol Anat Histol* 364(2):137-144, 1974.

The ubiquitous presence of blood group isoantigens in endothelial cells of blood vessels and erythrocytes was used in order to identify cells of endothelial origin in the course of morphological studies in angioblastic meningiomas and hemangioblastomas. Biopsy specimens of 70 cerebrospinal tumors coded as "angioblastic meningiomas" and 30 cerebellar hemangioblastomas were examined for blood group isoantigens using the specific red cell adherence (SRCA) test of Davidson (1972). In cerebellar and supratentorial hemangioblastomas a positive SRCA reaction in endothelial cells forming the prominent component of this neoplasm was opposed by a negative reaction in the large majority of interstitial (stroma) cells; this argues against their endothelial origin. Identical behavior in the SCRA test supports the histogenetic identity of hemangioblastomas and angioblastic meningiomas. A consistently negative SRCA reaction in cerebrospinal hemangiopericytomas, except for the endothelial cells lining the capillaries, supports the concept of a non-endothelial (pericytic) histogenesis of this neoplasm. Richly vascularized meningiomas with positive SRCA reactions restricted to vascular endothelial cells can be separated from angioblastic meningiomas, although transition forms are likely to occur. Of the 70 CNS tumors coded "angioblastic meningiomas," there were 10 hemangioblastomas, 11 hemangiopericytomas and 49 highly vascularized meningiomas. Angioblastic meningiomas suggested to originate from vasoformative elements (endothelium and pericytes) were found to represent 3.2 percent of a biopsy sample of 660 cases with cerebrospinal meningiomas. Their relationship to primary meningeal neoplasms needs further elucidation.

6890 INHIBITION OF RAT LYMPH NODE CELL CYTOTOXICITY BY HEPATOMA-ASSOCIATED EMBRYONIC ANTIGEN.

(E.) Rees, R. C. (Cancer Res. Campaign Lab., Univ. Nottingham, England), M. R. Price, R. W. Baldwin and L. P. Shah. *Nature* 252(5485):751-753, 1974.

The capacity of hepatoma-associated embryonic antigen (EA) to interfere with lymphocytotoxic reactions *in vitro* was investigated. Pretreatment of 5 x 10^5 lymph node cells (LNC) from multiparous (MP) rats with EA (12.5 μg) from hepatoma D23 reduced their cytotoxicity for D23 cells from 75 to 3%, representing a 96% inhibition of cytotoxicity. At concentrations which inhibited MP LNC cytotoxicity, D23 EA did not affect the cytotoxicity of tumor-immune LNC. These findings are further evidence that the tumor-specific antigen present on these aminoazo dye-induced tumors, and against which tumor-immune LNC exert their cytotoxic effect, is a distinct component from the tumor-associated EA. Pretreatment of MP LNC with EA (40-50 μg) from hepatoma D30 significantly inhibited their cytotoxicity for D23 target cells, indicating a cross reaction between hepatoma D23 and D30 EA.

6891 α_1-ANTITRYPSIN IN CIGARETTE SMOKERS.

(E.) Elson, L. A. (Inst. Cancer Res., Sutton, England), T. Betts and D. A. Darcy. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973,* 1:151-153, 1974.

The effect of cigarette smoking on serum levels of α_1-antitrypsin factor is described. The concentration of α_1-antitrypsin factor was measured by a quantitative gel-diffusion assay with goat anti-α_1-antitrypsin factor serum in the center well. The value for pooled serum from 25 male and 25 female non-smoking healthy volunteers was established as the 100% standard and values for 19 female and 14 male non-smokers and 18 male and 14 female smokers were related to the standard. Non-smokers were 99.6% of the standard and smokers were 117% of the standard, these mean values being statistically different. This preliminary clinical result confirmed findings from previous animal studies, using another serum globulin as marker. It is suggested that further human investigation is warranted because of known inherited variation in α_1-antitrypsin factor response to inflammation in man and variations in the composition of tobacco.

6892 FERRITIN, A HODGKIN'S DISEASE ASSOCIATED ANTIGEN.

(E.) Eshhar, Z. (Harvard Med. Sch., Boston, Mass.), S. E. Order and D. H. Katz. *Proc Natl Acad Sci USA* 71(10):3956-3960, 1974.

Antigens which exist in high frequency in tumor tissues of patients with Hodgkin's disease were obtained in relatively concentrated form by gel chromatography procedures. Further purification and analysis of these antigens demonstrated that the antigen of fast electrophoretic mobility (F-antigen) is normal tissue ferritin. The F-antigen contained iron and was similar to ferritin in molecular wt, amino-acid composition, electrophoretic mobility, isoelectric distribution, and immunological reactivity. Absorption of a monospecific heterologous anti-F serum with normal tissue ferritin completely removed all anti-F antibody activity. Immunoabsorptions were also performed with a polyspecific heterologous antiserum to crude Hodgkin's extracts, the antiserum containing antibodies reacting with F-antigen, the slower migrating S-antigen, and a third specificity present in lysates of normal peripheral blood lymphocytes (PL-antigen). Absorption of this serum with ferritin removed only anti-F activity, thus distinguishing the S- and PL-antigens from ferritin. The existence of ferritin in high quantities in serum of Hodgkin's disease patients may provide a tool of potential diagnostic and prognostic importance in the management of this disease.

6893 ANTIGENIC CHARACTERISTICS OF MURINE LEUKEMIA INDUCED BY CHEMICAL CARCINOGENS.

(E.) Colnaghi, M. I. (Natl. Cancer Inst., Milan, Italy) and G. Della Porta. *Bibl Haematol* (39):595-602, 1973.

The antigenicity of thymic lymphosarcomas was characterized in male and female mice of C3Hf, C57BL, and SWR strains. Ten day old animals received five 1 mg/g i.p. injections of urethane (1 injection/day) on alternate days to produce thymic lymphosarcomas. Lymphomas were kept by transplantation in preirradiated syngeneic hosts and were at no more than the fifth passage when first used. Mice were immunized using 5-6 inocula of a cell suspension prepared from either a single or multiple (2-3) tumors. Irradiated (10,000 r) or blocked (mitomycin 250 μg/10 x 10^6 cells) tumor cells were used for syngeneic immunization, while allogenic immunizations used both treated and untreated cells. Membrane fluorescence, trypan blue exclusion and ^{51}Cr-release *in vitro* tests were used to measure immune response, considering only cell surface antigenicity. Sera were always syngeneic to the target cell (no alloantigens) and were used undiluted, after inactivation. Syngeneic immunizations always yielded a negative cytotoxic or fluorescence index; allogeneic immunizations gave positive indices, as did blocked tumor cells. An effective immunity from humoral and cell-mediated responses was obtained when immunized and immunizing strains differed in the H-2 alleles. To demonstrate the cross-reacting tumor-specific antigenicity, C57BL anti K36 antiserum absorptions were conducted. Seven lymphomas completely absorbed the cytotoxicity activity of the antiserum. The results show that the urethane-induced lymphomas possess the Gross virus-associated antigenicity. Further investigation of the Gross cell surface antigen in the urethane-induced lymphomas and the antigen detected by the antisera from allogeneic immunizations is suggested.

6894 IMMUNOCHEMICAL STUDIES OF THE GROUP-SPECIFIC ANTIGENS OF MAMMALIAN C-TYPE VIRUSES. *(E.)*

Gilden, R. (Flow Lab., Inc., Rockville, Md.), D. Bova and S. Oroszlan. *Bibl Haematol* (39):800-809, 1973.

Purification methods for the major group-specific (gs) protein of four mammalian C-type viruses, together with evidence of assignment of both species-specific and interspecific cross-reactive determinants to the same highly purified structure are reviewed. The major internal virion protein of ultracentrifugally-isolated C-type RNA viruses was purified by isoelectric focusing of Tween 80-ether disrupted virus. This protein has an isoelectric point of 6.7 (mouse), 6.9 (hamster), 8.3 (cat), and 8.6 (rat). In SDS-polyacrylamide gels this protein corresponded to the third fastest migrating virion polypeptide and had a calculated molecular weight of 25,000-35,000. Evidence for the presence of both species-specific and interspecific determinants on the major gs proteins was provided by different experiments involving purification, heat inactivation, absorption, or precipitation. The interspecific cross-reactive determinants were not detected in non-mammalian C-type viruses. The species-specific determinants are those generally termed group-specific (gs) and are shared by all isolates from the homologous species.

6895 NEUTRALIZATION, IMMUNOFLUORESCENCE AND COMPLEMENT FIXATION TESTS IN IDENTIFICATION OF ANTIBODY TO A HERPESVIRUS TYPE 2-INDUCED, TUMOR-SPECIFIC ANTIGEN IN SERA FROM SQUAMOUS CERVICAL CARCINOMA. *(E.)*

Aurelian, L. (Sch. Med., Johns Hopkins Univ., Baltimore, Md.), J. D. Strandberg and R. L. Marcus. *Prog Exp Tumor Res* 19:165-181, 1974.

Eleven human sera used in this study were obtained from four patients with cervical carcinoma and from seven control subjects and were used to investigate the presence of virus-specific antibody other than neutralizing one, to determine whether such antibody is tumor-specific, and to establish a correlation between the presence of such antibody and the extent of the tumor. Sera obtained from 21 patients with untreated cervical neoplasia, from 21 patients with cervical carcinoma treated by radiation or hysterectomy, and from 21 controls with a negative history of cervical carcinoma or abnormal Papanicolaou smears were used in complement fixation tests only. The presence of neutralizing antibody to herpes simplex virus type-2 (HSV-2) did not correlate with cervical cancer in agreement with previous reports. Membrane fluorescence was observed at four and 24 hr after infection of cells with HSV-2 and staining with the sera previously used in neutralization. When individual sera were studied, complete correlation between the presence of neutralizing and fluorescent antibody was not observed. Presence of immunofluorescent antibody did not correlate with cervical cancer. Whereas all sera did not fix complement with the control antigen, all cancer sera (100%) and one control serum (14%) fixed complement with an antigen designated as AG-4, suggesting that antibody to AG-4 is virus-induced and tumor specific. The prevalence of antibody to AG-4 was significantly greater among women with invasive carcinoma of the cervix (90%) than among matched controls. Nineteen/21 women with neoplasia had antibody to AG-4 as compared to 2/21 matched controls. Sera from patients treated prior to blood collection did not have antibody to AG-4; antibody to HSV-2 was present in 100% of treated patients. It is suggested that this antigen may be a virion antigen of the inner capsid, a non-structural virus-coded antigen, or a cell-coded antigen induced by infection with HSV-2, similar or identical to the carcinoembryonic antigen associated with colon cancer. It is concluded that the data do not prove that HSV-2 causes cervical cancer.

6896 DISTRIBUTION OF FAST- AND SLOW-MIGRATING HODGKIN'S TUMOR-ASSOCIATED ANTIGENS.

(E.) Order, S. E. (Harvard Med. Sch., Boston, Mass.), J. Colgan and S. Hellman. *Cancer Res* 34(5):1182-1186, 1974.

The relative distribution of slow-migrating (S) antigen and fast-migrating (F) antigen in a variety of tissues and in lymphocytes from Hodgkin's disease patients was investigated using separation by discontinuous albumin gradients in order to determine the possible pathological significance of these antigens. Splenic or nodal tissue was obtained from patients undergoing biopsy or splenectomy, as well as part of the staging laparotomy in patients with Hodgkin's disease (one exception was a reticulum cell sarcoma specimen obtained postmortem). Aliquots of 1 x 10^9 cells were prepared fresh for bovine serum albumin (BSA) density gradient segregation. Aliquots of 1 x 10^8 cells were frozen for use in immunoelectrophoresis. Centrifugation at 900 rpm for ten min at 4 C produced a supernatant for antigenic analysis. Hodgkin's tumor infiltrate (4 x 10^8 cells/ml) was frozen for use in immunization. The S and F antigens were present in 35/36 Hodgkin's tumor infiltrates. A third specificity was identified, PL antigen, which is present on peripheral lymphocytes of both normal patients and patients with Hodgkin's disease. There was no difference between the percentage of cells recovered from the layers in the involved and uninvolved splenic tissue. Immunoelectrophoretic analysis of the nonsegregated cell suspensions of tumor infiltrates in six specimens revealed all to be positive in comparison to uninvolved spleens. F antigen was found in high concentrations in Hodgkin's disease infiltrates, which suggests the relationship of lymphoproliferation and the appearance of F antigen. It also appears in fetal hematopoietic tissue, which supports the concept that rapid proliferation of primitive hematopoietic cells expresses the same protein. S antigen was present in Hodgkin's infiltrates, malignant lymphocytic thymoma and reticulum cell sarcoma; its presence in neonatal thymus and its identification in Hodgkin's disease with antithymus antiserum demonstrates its T-cell relationship. It is suggested that studies be initiated to determine what stimuli may cause F and S antigenic expression in nodal and splenic lymphocytes.

6897 SHARED ANTIGENS BETWEEN *MYCOBACTERIUM BOVIS* (BCG) AND NEOPLASTIC CELLS. *(E.)* Minden, P. (Natl. Jewish Hosp. Res. Cent., Denver, Colo.), J. K. McClatchy, M. Wainberg and D. W. Weiss. *J Natl Cancer Inst* 53(5):1325-1331, 1974.

The possibility that *Mycobacterium bovis* and some neoplastic cells have common antigenic components was investigated using rabbits immunized with *Mycobacterium bovis* sonicates or with line 10 (transplantable guinea pig hepatocarcinoma cells). Other rabbits were immunized with line-1 cells, a tumor with antigenic characteristics different from those of line-10. The binding of antibodies in these sera to soluble radiolabeled antigens prepared from *Mycobacterium bovis* and line-10 cells was studied by precipitation of radiolabeled antigen-antibody complexes with antirabbit immunoglobulin. Antibodies in sera from rabbits immunized with *Mycobacterium bovis* and line-10 cells bound both the labeled *Mycobacterium bovis* and line-10 antigens. Antibodies in sera from rabbits immunized with line-1 cells bound the line-10 but not the *Mycobacterium bovis* antigen. Inhibition studies confirmed the specificity of these reactions. Inhibition experiments also showed that antigenic components are shared by *Mycobacterium bovis* and extracts of Rous sarcoma cells but not by extracts of a mouse plasma-cell tumor. Whether the antigens shared by *Mycobacterium bovis* and line-10 tumor cells are identical with tumor-specific antigens and whether they have a function in the outcome of immunotherapy remain open questions. The following interpretation of these observations is proposed: 1) antiline 10 and anti-line-1 show certain populations of antibodies stimulated by tumor cell antigens, some of which may be tumor specific and some of which, such as those stimulated by the embryonic antigens shared by these cells, are not 2) anti-line-10 and *Mycobacterium bovis* have some antibodies that were stimulated by antigens common to tumor cells and by *Mycobacterium bovis* 3) the antibodies present in both anti-line-10 and anti-line 1 are different from those shared by anti-line-10 and anti-*Mycobacterium bovis* 4) antigenic components are shared by *Mycobacterium bovis* and line-10 tumor cells, but not by *Mycobacterium bovis* and line-1.

6898 PROTECTION AGAINST GUINEA PIG HEPATOMAS BY PRETREATMENT WITH SUBCELLULAR FRACTIONS OF *MYCOBACTERIUM BOVIS* (BCG). *(E.)* Minden, P. (Natl. Jewish Hosp., Res. Ctr., Denver, Colo.), M. Weinberg and D. W. Weiss. *J Natl Cancer Inst* 52(5):1643-1645, 1974.

The protective activities of the methanol-extraction residue (MER) of killed myobacterium bovis (BCG) and of sonically disrupted, heat-killed culture of BCG (BCG-SS) against line-10 hepatoma were studied in young adult male and female guinea pigs. For the MER, single intradermal injection of 0.5 or 1.0 mg in 0.1 ml physiologic saline was given into the upper abdominal area. The animals were then challenged id with 10^6 hepatoma cells at a separate site in the abdominal area 27, 42, or 58 days later. For the BCG-SS, intradermal injections containing 25 or 50 µg nitrogen (N) in 0.1 ml were given 27 days before challenge. Small papules developed in all animals at the site of MER injections. These reached a mean diameter of 11 mm after 4 days, thereafter becoming smaller and barely detectable by day 10. Following BCG-SS, the initial swellings gradually disappeared by 48 hours. Pretreatment with MER or BCG-SS did not permit the neoplastic lesions of some animals to enlarge after 10 days and the lesions gradually diminished until they were no longer visible by about day 20. If the lesions did not begin to regress by days 10-14, their subsequent courses were similar to the controls. Seven of the 17 animals that received MER and 4 of the 6 given BCG-SS were protected. There were no dosage effects of MER, but 50 µg of BCG-SS may have been superior to 25 µg N. In untreated control animals the local lesions induced by the challenge injections grew and became necrotic, the regional lymph nodes enlarged visibly, and all such animals died with widespread metastases in 60-70 days. It is concluded that the mode of action of the supposedly nonspecific antitumor activities of living BCG and of certain isolated constituents for some BCG strains differs, and there is a need for a comparative evaluation of the prophylactic and therapeutic potentials of mycobacterial entities in tumor systems.

6899 DIFFERENCE IN MICROVISCOSITY INDUCED BY DIFFERENT CHOLESTEROL LEVELS IN THE SURFACE MEMBRANE LIPID LAYER OF NORMAL LYMPHOCYTES AND MALIGNANT LYMPHOMA CELLS. *(E.)* Shinitzky, M. (Lab. Membr. Bioregul., Weizmann Inst. Sci., Rehovot, Israel) and M. Inbar. *J Mol Biol* 85(1):603-615, 1974.

Microviscosity in the surface membrane lipid layer of normal rat and mouse lymphocytes and malignant murine lymphoma cells, and in liposomes prepared from their lipid extracts, was determined using the fluorescence polarization properties of 1,6-diphenyl-1,3,5-hextriene embedded in the membrane lipid layer. The microviscosity values in both the intact cells and in the liposomes were distinctly greater for the normal lymphocytes than for the lymphoma cells, whereas the fusion activation energy in both cell types and their liposomes was 8 kcal/M. The relative cholesterol concentration in the lymphoma cells was about half that in the normal lymphocytes, a difference which might account for the difference in fluidity. This conclusion is supported by the observed changes in microviscosity, which followed artificial changes in the cholesterol concentrations in the surface membranes of both cell types. Introduction of exogenous cholesterol into the cell surface membranes was performed with lecithin-cholesterol (1:1) liposomes, and in the lymphoma cells resulted in an increase in microviscosity to the level of normal lymphocytes. Extraction of native cholesterol from the cell surface membranes *via* lecithin liposomes resulted in a decrease in microviscosity to the level seen in the lymphoma cells. By virtue of its control over the microviscosity of the lipid layers, the level of cholesterol in cell surface membranes may play an important role in determining the biological activities of normal and malignant cells.

6900 LYMPHOCYTE CYTOTOXIC ACTIVITY ON EMBRYONIC COLON TARGET CELLS IN PATIENTS WITH CARCINOMA OF THE COLON.

(E.) Onody, K. (Natl. Inst. Hematol. Blood Transfus., Budapest, Hungary), A. Kovacs and G. Petranyi. *Neoplasma* 21(3):291-295, 1974.

The cytotoxicity of serum or lymphocytes from patients with colon cancer or ulcerative colitis was studied using cultured embryonic colon cells. The cultured cells were used as target cells in order to avoid contamination with extraneous gut antigens. Release of ^{51}Cr from labeled target cells was used as the cytotoxicity index ($\bar{x}i$) defined as: (radioactivity of sample minus spontaneous release)/(total radioactivity minus spontaneous release). Index values greater than 0.25 were considered evidence of cytotoxicity. Lymphocytes from ten colon cancer patients, ten ulcerative colitis patients and 18 healthy controls gave $\bar{x}i$ values of 0.09, 0.17, and 0.15 resp. There was no evidence of cytotoxicity reversal in the further presence of carcinoma serum or serum plus complement (no lymphocytes). It is concluded that colonic cancer does not produce cytotoxic circulating antibody or blocking antibody but carcinoembryonic antigen activity is not precluded since it is probably an autoimmune antigen which would not be expected to produce a cytotoxic reaction.

6901 LYMPHOCYTE REACTIVITY TO PHYTOHAEMAGGLUTININ AND ALLOGENEIC LYMPHOCYTES IN 32 UNTREATED PATIENTS WITH HODGKIN'S DISEASE.

(E.) Lang, J. M. (Univ. Hosp. Cent., Strasbourg, France), F. Oberling, P. Bigel, S. Mayer and R. Waitz. *Biomedicine* 21(9): 372-377, 1974.

Two *in vitro* cell immunity tests, lymphocyte response to phytohemagglutinin (PHA) and mixed lymphocyte reaction (MLR), were evaluated in 32 untreated patients with Hodgkin's disease. Forty healthy controls were used for the PHA test only. Twenty-two patients (at different clinical stages) out of 29 (76.8%) exhibited positive reactions to both tests; one reacted negatively to PHA and seven others were negative to MLR. The fact that there were more negative reactions in the MLR test than in the PHA test suggested that the former might be a more sensitive assay for the transformation capacity of lymphocytes in Hodgkin's disease patients. Results of the PHA test in the control group showed a wide range of positive values, but were significantly higher than in the patient group ($p < 0.001$). In neither technique, however, could significant differences be found which could be related to the clinical stage of the disease, to the presence or absence of systemic symptoms, or to the histopathology.

6902 IS THERE A FACTOR IN SERA FROM CANCER PATIENTS THAT INHIBITS LYMPHOCYTE RESPONSE TO PHYTOHAEMAGGLUTININ?

(E.) Whitehead, R. H. (Welsh Natl. Sch. Med., Cardiff), P. M. Bolton and R. G. Newcombe. *Eur J Cancer* 10(12):815-818, 1974.

To determine whether the diminished phytohemagglutinin (PHA) response of lymphocytes from cancer patients is due to a defect in the lymphocytes or to the presence of an inhibitor in the serum, lymphocytes from patients with benign breast disease (controls), early breast cancer, and advanced breast cancer were stimulated by PHA in the presence of autologous serum, human AB serum, pooled sera from patients with advanced breast cancer, pooled sera from patients with advanced colon cancer, or fetal bovine serum. All non-self sera caused significant depression of PHA stimulation in the control lymphocytes, while the PHA response of the lymphocytes from the patients with early breast cancer was significantly depressed only in the presence of fetal calf serum, human AB serum, and the pooled sera from patients with advanced colon cancer. The lymphocyte response to PHA in the patients with advanced breast cancer was significantly depressed only by the pooled advanced colon cancer sera. Only one subject stimulated less well in autologous serum than in any other serum; this patient had undergone bilateral adrenalectomy and was receiving cortisone acetate and fluodrocortisone daily. The levels of alpha-2-macroglobulin in the allogeneic sera were within normal limits, but the levels of alpha-2 globulin in the pooled breast cancer and colon cancer sera were abnormally high. The results favor the concept that the depression of the lymphocyte response to PHA in the presence of cancer serum is a nonspecific effect of nonself serum. Depression of the lymphocyte response to PHA in cancer patients may be due to a primary or secondary lymphocyte defect.

6903 EARLY EVENTS IN LYMPHOCYTE TRANSFORMATION BY PHYTOHEMAGGLUTININ. III. INHIBITION OF RNA SYNTHESIS AND TRANSFORMATION BY CORDYCEPIN.

(E.) Pogo, B. G. T. (New York City Public Health Res. Inst., N.Y.). *Cell Immunol* 14(1):134-138, 1974.

The adenosine analog cordycepin, an inhibitor of RNA synthesis, was studied to determine if it inhibits phytohemagglutinin transformation of lymphocytes. Exposure of phytohemagglutinin-treated lymphocytes to cordycepin (1-10 μg/ml) for 48 hr reduced subsequent DNA synthesis by as much as 75% compared to phytohemagglutinin alone. Inhibitory effects of cordycepin were even more pronounced on phytohemagglutinin-induced RNA synthesis, measured by ^{3}H-uridine incorporation. Inhibition was greatest during the first two hr of exposure to cordycepin, and more pronounced (greater than 50% inhibition) with brief exposure (five min) to labeled uridine than with 30 min exposure. RNA polymerase activity, measured *in vitro*, and protein synthesis in cells exposed to phytohemagglutinin (with and without cordycepin) for the first four hr were not significantly different. It is concluded that cordycepin inhibits the rapid mRNA assembly step rather than the DNA-dependent RNA polymerase step.

6904 RESULTS OF THE MACROPHAGE MIGRATION TEST DURING THE EARLY STAGES OF RAUSCHER'S LEUKEMIA. *(Ger.)* Komitowski, D. (German Inst. Cancer Res., Heidelberg) and J. Kurka. *Experentia* 30(6):648-649, 1974.

The macrophage migration test, developed by George and Vaughan, was performed during the early stages of Rauscher's leukemia in 8-10-wk-old female NMRI mice. The animals were inoculated i.p. with cell-free supernatant from homogenized leukemic spleen from the same strain. Rauscher's virus markedly inhibited the macrophage migration 24 hr after inoculation. This effect was even more pronounced when antigen was added to the culture. A first maximum on the third day was followed by a marked reduction of inhibition until the fifth day; complete cessation of the macrophage migration occurred between the 12th and 13th days. The marked inhibition on the third day must be attributed to specific immunological antigen stimulation of T-lymphocytes. The decrease in inhibition which occurred on the fifth day may either be due to specific immunological antigen stimulation of T-lymphocytes or to dynamic changes in the macrophages themselves.

6905 DIFFERENCES IN LECTIN AGGLUTINABILITY OF NORMAL AND TRANSFORMED CELLS IN INTERPHASE AND MITOSIS. *(E.)* Shoham, J. (Dep. Genet., Weizmann Inst. Sci., Rehovot, Israel) and L. Sachs. *Cold Spring Harbor Conf Cell Proliferation* 1:297-304, 1974.

Differences in surface fluorescence of normal and transformed cells in interphase and mitosis were correlated with the agglutinability of these cells and the number of concanavalin A (ConA) molecules bound to the cell surface in nonsaturation conditions. The cells studied were normal hamster cells, hamster cells transformed *in vitro* by Rous sarcoma virus, polyoma virus, or dimethylnitrosamine, and 3T3 cells transformed by simian virus 40 or doubly transformed by SV40 and polyoma virus. ConA and wheat germ agglutinin agglutinated normal hamster cells in mitosis but not in interphase. Transformed hamster and 3T3 cells were agglutinated by these lectins in interphase but not in mitosis. Soybean agglutinin agglutinated transformed hamster cells in interphase but not in mitosis; in no case were hamster cells agglutinated by soybean agglutinin. When cells were incubated with fluorescein-conjugated ConA (F-ConA) (1 and 2.5 μg), the highest percentage of fluorescent cells was found among transformed interphase and normal mitotic cells, the next highest with transformed mitotic cells, and the least with normal interphase cells. Comparison of ^{3}H-ConA and F-ConA binding indicated that 1000 molecules/μ^2 cell surface are needed for the appearance of surface fluorescence in transformed interphase and normal mitotic cells, 2000 molecules for transformed mitotic cells, and 4000-5000 molecules for normal interphase cells. The rate of surface fluorescence acquisition by transformed mitotic cells was lower than that for transformed interphase cells. The results indicate that the number of ConA binding sites on the cell surface and their affinity for ConA does not differ among these cells.

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6909 CELL-MEDIATED AND HUMORAL IMMUNE RESPONSE TO ACUTE LEUKEMIA CELLS AND SOLUBLE LEUKEMIA ANTIGEN-RELATIONSHIP TO IMMUNOCOMPETENCE AND PROGNOSIS. *(E.)* Gutterman, J. U. (Baylor Coll. Med., Houston, Tex.), E. M. Hersh, G. Mavligit, E. J. Freireich, R. D. Rossen, W. T. Butler, K. B. McCredie, G. P. Bodey, Sr. and V. Rodriguez. *Recent Results Cancer Res* 47:97-112, 1974.

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6949 DETECTION OF TUMOUR-ASSOCIATED TRANSPLANTATION ANTIGEN OF BO-IV CARCINOMA OF SOMATIC CELLS OF TUMOUR-FREE SYNGENEIC MICE. III. DEMONSTRATION OF THIS ANTIGEN ON THE LYMPHORETICULAR SYSTEM OF ADULT MICE. *(E.)* Rejthar, A. (Fac. Med., J. Ev. Purkyne Univ., Brno, Czechoslovakia) and J. Wotke. *Folia Biol (Praha)* 20(4):244-251, 1974.

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6952 ISOLATION OF A SPECIFIC PLASMACYTOMA TUMOUR ANTIGEN, REACTIVE BOTH IN HUMORAL AND CELL MEDIATED IMMUNITY. *(Fr.)* Kolb, J. P. (Inst. Sci. Res. Cancer, Villejuif, France), G. Lespinats and M. F. Poupon. *Ann Immunol (Paris)* 125(5):835-845, 1974.

6953 SELECTIVE INDUCTION OF CELL-MEDATED IMMUNITY AGAINST AN SV40 VIRUS INDUCED TUMOR IN THE HAMSTER. *(E.)* Hunter, R. (Dept. Pathol., U. Chicago, Ill.) and F. Strickland. *J Reticuloendothel Soc* 15(6):12a-13a, 1974.

6954 SURFACE PROPERTIES OF CELLS DIRECTLY OBTAINED FROM HODGKIN'S DISEASE (HD) LESIONS. *(E.)* Braylan, P. C. (Natl. Cancer Inst., Natl. Inst. Health, Bethesda, Md.), E. S. Jaffe and C. W. Berard. *J Reticuloendothel Soc* [*Suppl*] 16:45a, 1974.

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6956 DELAYED LYMPHOMA FORMATION IN IMMUNOSUPPRESSED NZB/NZW MICE TREATED WITH TILORONE HCl. *(E.)* Walker, S. E. (Dept. Med., U. Michigan, Ann Arbor) and M. R. Anver, *Clin Res* 22(4):612A, 1974.

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6958 DNA-SYNTHESIZING MYELOID AND LYMPHOID CELLS IN THE BLOOD OF HAEMODIALYSIS PATIENTS AND KIDNEY GRAFT RECIPIENTS. *(E.)* Virolainen, M. (3rd Dep. Pathol., Univ. Helsinki, Finland), M. Lalla, A. Pasternack, C. Wasastjerna and P. Hayry. *Scand J Haematol* 12(5):391-400, 1974.

6959 SUSCEPTIBLE AND NON SUSCEPTIBLE PHENOTYPES OF MOPC 173 PLASMOCYTOMA TO THE KILLING EFFECT OF CONCANAVALIN A: STUDY ON THE CANCANAVALIN A BINDING SITES. *(E.)* Zachowski, A. (Inst. Biol. Mil., C.N.R.S., Univ. Paris, France), B. Prigent, M. Monsigny and A. Paraf. *Biochimie* 56(11-12):1621-1625, 1974.

6960 CHRONIC LYMPHOCYTIC LEUKEMIA OF T-CELL ORIGIN: DEMONSTRATION IN TWO CASES BY THE USE OF ANTI-THYMOCYTE MEMBRANE ANTISERUM. *(E.)* Yodoi, J. (Fac. Med., Kyoto Univ., Japan), K. Takatsuki, N. Aoki and T. Masuda. *Acta Haematol Jpn* 37(3):289-292, 1974.

6961 NONSPECIFIC IMMUNE RESPONSES IN HEMATOSARCOMAS AND ACUTE LEUKEMIAS. *(E.)* Schneider, M. (Paul-Brousse Hosp., Villejuif, France), G. Mathé, L. Schwarzenberg, P. Pouillart, R. Weiner, J. L. Amiel, M. Hayat, C. Jasmin and F. de Vassal. *Recent Results Cancer Res* 47:42-53, 1974.

6962 DELAYED-HYPERSENSITIVITY REACTIONS IN PATIENTS WITH CANCER. *(E.)* Pinsky, C. M. (Cornell Univ. Med. Coll., N. Y.), A. El Domeiri, A. S. Caron, W. H. Knapper and H. F. Oettgen. *Recent Results Cancer Res* 47:37-41, 1974.

6963 RECALL OF TUMOUR RESISTANCE IN LONG-TERM *SALMONELLA ENTERITIDIS* 11RX IMMUNISED MICE. *(E.)* Ashley, M. P. (Dep. Microbiol., Univ. Adelaide, Australia), I. Kotlarski and D. Hardy. *Aust J Exp Biol Med Sci* 52(6):859-871, 1974.

6964 CHROMOSOMES OF GOLDEN HAMSTER TUMORS OBTAINED BY TUMORAL POLYPASSAGE OF THE RS 1 RAT TUMOR. *(E.)* Encut, I. (Oncol. Inst., Bucharest, Rumania), L. Cioloca and F. Liciu. *Tumori* 60(4):289-292, 1974.

6965 CHARACTERISTICS OF LYMPHOCYTES INFILTRATING HUMAN BREAST CANCERS. *(E.)* Richters, A. (Dept. Pathol., Univ. Southern California, Los Angeles). *J Reticuloendothel Soc* 16 (Suppl.):8a, 1974.

6966 DIFFERENCES IN CELLULAR SURFACE CHARGE PROPERTIES OF NORMAL AND SIMIAN VIRUS 40 TRANSFORMED MOUSE 3T3 FIBROBLASTS. *(E.)* Tenforde, T. (Donner Lab., Univ. California, Berkeley), J. Risius and C. A. Tobias. *Fed Proc* 33(5):1416, 1974.

6967 ISOLATION OF A RECEPTOR ASSOCIATED WITH CHICKEN SUSCEPTIBILITY TO ONCOGENIC VIRUS. *(E.)* Desai, P. R. (Evanstown Hosp., Ill.), G. F. Springer and I. Banatwala. *Fed Proc* 33(5):1416, 1974.

6968 ELECTRON MICROSCOPIAL STUDIES ON THE SURFACE OF NORMAL AND TRANSFORMED CELLS. *(E.)* Moretz, R. C. (Univ. Colorado, Boulder). *Fed Proc* 33(5):1416, 1974.

See also:

* (Rev): 6632, 6634, 6635, 6636, 6637, 6638, 6639, 6640, 6641, 6652, 6658, 6672, 6679, 6683
* (Chem): 6711, 6735, 6736
* (Phys): 6769
* (Viral): 6800, 6810, 6814, 6821, 6830
* (Path): 6983

6969 LUTEOTROPIN ADENOMAS. CONCERNING NINE CASE STUDIES. *(Fr.)* Tourniaire, J. (Endocrinol. Clin., Lyon Univ., France), P. Guinet, I. Bachelot, G. Pousset, D. Pallo, G. Sassolas, J. Trouillas, and M. L'Hermite. *Rev Otoneuroophthalmol* 46(3):303-320, 1974.

Observations of nine cases of luteotropin adenomas in women 17 to 35 yr old are presented. The clinical picture includes amenorrhea, primary in one case and secondary in all other cases. This is associated with patent or latent galactorrhea in all but one case. The disease is characterized by elevated plasma concentration of luteotropin. The effects on other functions of the anterior pituitary are irregular. Luteotropin adenoma may be caused by a disorder of the hypothalamus, or it may be a primary adenoma of lactotropic cells. The literature on luteotropin adenoma is reviewed with respect to laboratory studies of luteotropin plasma concentration, other anterior pituitary functions, and induced hyperglycemia; treatment; and nosological complexities.

6970 ONCOCYTOMA, A NEW HISTOLOGICAL TYPE OF HYPOPHYSEAL ADENOMAS. *(Fr.)* Landolt, A. M. (Clin. Neurosurg., Univ. Zurich, Switzerland). *Rev Otoneuroophtalmol* 46(3):265-268, 1974.

A case of hypophyseal adenoma of a new histological type was studied in detail. The tumor was successfully treated surgically at first, and a recurrence 13 years later was also excised successfully. Electron microscopy revealed that the tumor was composed exclusively of oncocytes. It seemed to have resulted from autonomous growth of cells with altered metabolism, rather than from a secondary transformation of a preexisting adenoma, since no intermediate stages of the transformation were found. The extremely slow growth of the tumor, resulting in recurrence of symptoms after a delay of 13 years, supports the hypothesis that oncocytes are, in part, degenerated cells.

6971 INVOLVEMENT OF CHROMOSOMES 8, 9, 19 AND 22 IN PH^1 POSITIVE AND PH^1 NEGATIVE CHRONIC MYELOCYTIC LEUKEMIA IN THE CHRONIC OR BLASTIC STAGE. *(E.)* Gahrton, G. (Karolinska Inst., Stockholm, Sweden), J. Lindsten and L. Zech. *Acta Med Scand* 196 (5):355-360, 1974.

Chromosomes from bone marrow and blood samples taken from 17 patients with chronic myelocytic leukemia were examined using the Q-banding technique. Eight of the patients were studied before initiation of busulfan treatment and the rest at a stage of insufficient treatment. Fourteen patients studies were at a chronic stage and were Ph^1 positive. Two patients who were Ph^1 positive were in the blastic stage of the disease as was one Ph^1 negative patient. In 15 of the Ph^1 positive patients, extra chromosomal material was found on the end of the long arm of chromosome 9. The other Ph^1 positive patient had additional material on chromosome 19. In all 16 Ph^1 positive patients, chromosome 22 lacked material which was similar to that seen on chromosomes 9 and 19. No additional chromosomal material was observed on any chromosome in the Ph^1 negative patient. Two of the three Ph^1 positive patients in the blastic stage had extra chromosomes. It is suggested that translocations between chromosomes 19 and 22, and 9 and 22 may have occurred. The extra number 8 chromosomes (seen in two of the three Ph^1 positive patients in the blastic stage) in hematopoietic cells appears to be associated with hematological malignancies, particularly with those which have a potential for being transformed to a myeloblastic type of leukemia.

6972 IDENTIFICATION OF CHROMOSOME ANOMALIES IN THE BLASTIC PHASE OF CHRONIC MYELOCYTIC LEUKEMIA (CML) BY GIEMSA- AND QUINACRINE-BANDING TECHNIQUES. *(E.)* Hossfeld, D. K. (Tumor Res., Univ. Clin. Intern. Med., Essen, West Germany). *Humangenetik* 23(2):111-118, 1974.

The chromosome constitution of three cases of chronic myelocytic leukemia in the blastic phase, in which satisfactory chromosome banding patterns could be obtained, was described. The banding pattern was obtained by atebrin staining and the trypsin-saline-Giemsa method. In all three cases the formation of the Ph^1 chromosome appeared to be associated with a translocation of about half of the long arm of chromosome 22 onto the terminal part of the long arm of chromosome 9. Chromosome 9q+ appeared to be a translocation product. There is evidence that the deleted segment of chromosome 22 is not invariably translocated to chromosome 9. A Ph^1+ chronic myelocytic leukemia is proposed with a translocation 2q+; 22q. In all three cases the two Ph^1 chromosomes were identical in size, centromere position and banding pattern. In all cases the supernumerary chromosome in group 19-20 was chromosome 19. The significance of this observation in the karyotypic evolution during the blastic phase of chronic myelocytic leukemia needs confirmation; however it is well known that in the blastic phase of chronic myelocytic leukemia additional chromosomes in group 19-20 occur next in frequency to additional chromosomes in groups 21-22 and 6-12. All three cases had supernumerary C-like chromosomes. Only one of them could be assigned with some certainty to one of the chromosome pairs of group 6-12. The other C-like chromosomes exhibited a banding pattern different from each other and from the normal members of group 6-12, and, hence, were designated marker chromosomes. The data may demonstrate the considerable advantage of interpreting chromosomal changes in leukemias when banding methods are applied.

6973 NON-RANDOM CHROMOSOME CHANGES IN HUMAN CANCER. *(E.)* Miles, C. P. (Dep. Pathol., Univ. Utah, Salt Lake City). *Br J Cancer* 30(1):73-85, 1974.

Karyotypes of 194 cells obtained from 21 patients (seven solid tumors, 14 cells in effusion) were made in order to determine the types of chromosomal abnormalities most frequently seen. The results indicated that chromosomal changes were not random in this series. The rankings of chromosome groups with respect to gains and losses in various types of cancer were determined. The 6-12+X group was found to have the most gains, and the 4-5 group the greatest deficit. The etiology of the apparent gains and losses in chromosome groups is discussed with respect to oncogenesis. It is suggested that the formation of new chromosome structures may be responsible for reported gains or losses in particular groups. Cancer karyotypes are postulated to evolve through a process of mutation and selection in which doubling of the chromosome complement occurs. Homologues with larger amounts of concentrated blocks of heterochromatin (i.e. late replicating DNA) seem more apt to be lost.

6974 GENERAL IMPLICATIONS OF CHROMOSOMAL ALTERATIONS IN HUMAN LEUKEMIA.

(E.) Trujillo, J. M. (M. D. Anderson Hosp. Tumor Inst., Houston, Tex.), M. J. Ahearn and A. Cork. *Hum Pathol* 5(6):675-686, 1974.

Sequential bone marrow cytogenetic studies were made in a series of 170 patients with acute leukemia. The incidence of aneuploidy was 40.5%. Most of these patients had acute myelogenous leukemia. Forty-six percent of the aneuploid karyotypes were hyperdiploid; 25% were hypodiploid and 20% were pseudodiploid. Certain of the aneuploid cytogenetic profiles had a tendency to occur in repetitive fashion in genetically unrelated patients. Certain profiles also carried prognostic implications. Patients with diploid and hyperdiploid karyotypes survived for 49 weeks (mean survival time), whereas those with pseudodiploidy and hypodiploidy survived for only 36 and 13 weeks, resp. These studies showed repeatedly that a favorable response to therapy is marked by early disappearance of the aneuploid or pseudodiploid clone and that this same abnormal clone reappears when the leukemia relapses. By contrast, in patients with chronic myelogenous leukemia in remission, the Ph^1 chromosome persists in the bone marrow at its initial frequency. These findings indicate that, when present, these chromosomal alterations define specifically the leukemic cells and that although the presence of an occasional aneuploid cell in the bone marrow probably does not have major significance, especially in older individuals, the existence of a well established pseudodiploid or aneuploid clone in the hematopoietic tissue is highly indicative of a neoplastic process. In conclusion, the contributions of cytogenetics in the study of human leukemia are reviewed and the clinical aspects of these studies are emphasized since cytogenetic parameters are now used widely in the clinical evaluation of leukemia.

6975 COEXISTENCE OF GASTRIC LEIOMYOMAS AND CHEMODECTOMAS.

(Pol.) Dolinski, J. (Med. Acad., Warsaw, Poland), L. Noczynski and J. Rabczynski. *Wiad Lek* 26(20):1917-1920, 1973.

Many leiomyomas and chemodectomas were found in the stomach of a 27-yr-old woman. The entire stomach was filled with tumors situated at both the greater and lesser curvatures and growing both into the gastric lumen and externally infiltrating the pancreas and greater omentum. The lymph nodes in the area were enlarged. The stomach was separated from the pancreas and gastric resection performed with simultaneous removal of the spleen and the greater and lesser omentum. Histologic examination revealed leiomyomas, chemodectomas and hyperplasia of the lymph nodes. The post-operative course was uneventful. It is suggested that the chemodectomas originated from the parasympathetic plexi of both vagus nerves.

6976 PHASE 1 AND PHASE 2 PROLIFERATIVE LESIONS OF COLONIC EPITHELIAL CELLS IN DISEASES LEADING TO COLONIC CANCER.

(E.) Lipkin, M. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.). *Cancer* 34(3/Suppl.):878-888, 1974.

The biosynthetic pathways involved in the proliferation of colon cells during neoplastic transformation were studied in three groups of subjects: 1) patients in the general population who developed isolated polyps, villous adenomas or carcinomas; 2) patients with familial polyposis; and 3) mice that had been given 1,2-dimethylhydrazine. Colonic epithelial cells in man began to develop some of the characteristics of malignant cells while they still appeared normal on conventional morphological examination. The cells passed through specific phases during which they expressed increasingly abnormal proliferative characteristics. As a Phase 1 proliferative lesion developed, colonic epithelial cells did not repress DNA synthesis during their maturation, and began to develop an enhanced ability to proliferate. The over-all kinetics of cell proliferation remained normal, without a net retention or accumulation of cells in the mucosa. As a Phase 2 proliferative lesion formed, the cells began to develop properties that enabled them to be retained in the mucosa in increasing numbers. The over-all kinetics of cell proliferation became abnormal, and net retention and accumulation of cells began to be observed. These changes were accompanied by differentiation-specific molecular errors, resulting in the abnormal persistence of metabolic pathways leading to enhanced DNA synthesis. These proliferative cellular lesions were seen in the three groups studied, suggesting that they are major steps on a final common molecular pathway leading to malignant transformation.

6977 "PRECANCER" LESIONS IN ULCERATIVE COLITIS. A RETROSPECTIVE STUDY OF RECTAL BIOPSY AND COLECTOMY SPECIMENS.

(E.) Yardley, J. H. (Johns Hopkins Univ. Sch. Med., Baltimore, Md.) and D. F. Keren. *Cancer* 34(3/Suppl.):835-844, 1974.

Colectomy specimens (from 41 patients) and rectal biopsies (412 biopsies from 204 patients with ulcerative colitis) obtained over an 11-yr period were assessed to: 1) provide an overview of precancer changes as they would be seen in a large unselected group of rectal biopsies from patients with ulcerative colitis; 2) help determine which criteria are most valid when attempting to identify precancer; and 3) test the over-all reliability of the rectal biopsy as a tool for detecting precancer. Seven out of eight resections containing carcinoma also showed flat or adenomatous mucosal lesions, characterized by the marked epithelial atypia, villous change, and reduced epithelial mucus content indicative of precancer. Similar precancerous changes were found in 3 out of 412 rectal biopsies. Two of these patients had subsequent colectomy with invasive carcinoma. The third had diffuse precancerous changes throughout the colon in the form of multiple adenomas. It is concluded that precancer changes are a valid means for identifying the population of ulcerative colitis patients at highest risk of developing carcinoma of the colon, and that some of these individuals can be discovered by using rectal biopsy. It was also clear, however, that active colitis artificially increased the number of biopsies showing changes suspicious of precancer. At the same time, bonafide cases of precancer involving other parts of the colon can go undetected by using only rectal biopsy. It is suggested that those patients might be more successfully identified by using colonoscopy, combined with biopsy at regular intervals in individuals with ulcerative colitis who are known on clinical grounds to be especially likely to develop carcinoma.

6978 HEREDITARY LYMPHOSARCOMA IN WH RABBITS AND HEREDITARY HEMOLYTIC ANEMIA ASSOCIATED WITH THYMOMA IN STRAIN X RABBITS. *(E.)* Meier, H. (Jackson Lab., Bar Harbor, Me.) and R. R. Fox. *Bibl Haematol* (39):72-92, 1973.

Genetic studies, pathological examinations and complement-fixation (CF) tests for C-type RNA tumor virus antigen were carried out in WH strain rabbits and X strain rabbits; the former strain is susceptible to lymphosarcoma, while the latter strain is susceptible to thymomas associated with hemolytic anemia. Both tumors were found to be conferred by susceptibility to single autosomal recessive genes designated ls (lymphosarcoma) and ha (thymoma associated with hemolytic anemia). No complete C-type particles were found using electron microscopy. Positive reactions to anti-sera produced in rats to murine C-type virus were seen in the WH strain. The authors suggest this to be tentative evidence of C-type RNA genome coding of the group specific antigen. It is suggested that the gene ha may be the same as gene ls, but that phenotypic expression is different due to the rest of the genotype.

6979 ON THE HISTOGENESIS OF MYELOID INFILTRATES IN LYMPH NODES IN ACUTE MYELOLEUKEMIA. *(Rus.)* Logoida, D. M. (Lvov Med. Inst., USSR). *Vrach Delo* (9):11-14, 1974.

Studies were conducted of 207 lymph nodes from 34 patients who died of acute myeloleukemia. Four stages of leukemic infiltration of the nodes were distinguished: (1) infiltration of the hilus; (2) the hilus and medullary cords; (3) medulla; (4) the entire node. Microscopic examination revealed leukemic infiltrates from oxidase-positive cells in 180 nodes (87%). Submaxillary, cervical, parapancreatic and lumbar nodes were most frequently affected. A direct correlation was observed between the number of leukocytes in the peripheral blood and the degree of leukemic infiltration of the lymph nodes. Treatment by prednisolone and 6-mercaptopurine had no effect on the degree of leukemic infiltration. In cases of moderate infiltration, the sinuses of lymphatic nodes were filled with lymph containing a great number of erythrocytes, few lymphocytes and a few oxidase-positive cells which were readily absorbed by reticular macrophages. No reticulo-endothelial cells were observed to transform into oxidase-positive leukocytes. The results bear out the contention that myeloid metaplasia of the lymph nodes originates in the cell tissue of the hilus and trabeculae, then spreading to the cortical follicles, while oxidase-positive cells reach the sinuses from the hyperplastic medullary cords or are brought there by lymph from numerous extravasates.

6980 CANCER OF THE COLON, RECTUM, AND ANUS: A REVIEW OF 2313 CASES. *(E.)* Falterman, K. W. (Louisiana State Univ. Sch. Med., New Orleans), C. B. Hill, J. C. Markey, J. W. Fox and I. Cohn, Jr. *Cancer* 34(3):951-959, 1974.

The classification and survival of 2313 large bowel cancer cases, admitted to Charity Hospital, New Orleans from 1948-1970 are presented. Features of this population are its ethnic composition (60% black and 40% white for cancer patients, 70% black and 30% white for total hospital admissions), mean age (63 yr), and higher incidence of cancer in females (58%). The malignant lesions were considered localized in 896, regional in 481 and distant or diffuse in 504 patients. The majority of lesions were in the sigmoid and rectal segments with the largest distribution in the most advanced stage (Dukes' classification, stage D, having distant metastases). Postoperatively, 1568 patients recovered successfully; 318 died following a first procedure, 38 after a second procedure and one after a third-stage procedure. Sixteen percent of all procedures performed resulted in an operative mortality. The procedure was classified as curative in 923 patients, and palliative in 628. Microscopically, most of the lesions were classified as adenocarcinoma. Overall five yr survival was 24%, which reflects the generally advanced stage of disease in this series of patients at the time of diagnosis. In cases deemed potentially curable, five yr survival was 47%. Better survival in females than males was consistently found. (10 references)

6981 RATE OF NUCLEIC ACID SYNTHESIS IN MAMMARY HYPERPLASTIC ALVEOLAR NODULE OUTGROWTHS AND TRANSPLANTED ADENOCARCINOMAS IN PREGNANT AND LACTATING MICE. *(E.)* Bartley, J. C. (Child. Hosp. Med. Cent., North. California, Oakland), S. Barber and S. Abraham. *Cancer Res* 34(10):2571-2575, 1974.

The *in vitro* synthesis of RNA and DNA was measured in normal tissues and in serially transplanted outgrowths of hyperplastic alveolar nodules and adenocarcinomas of mammary glands of pregnant and lactating C3H/Crl mice. The rate of DNA synthesis in normal glands of mice at midlactation was only 12% of that in glands from mice in late pregnancy. A decrease of 40% in the rate of RNA synthesis was observed in the glands during transition from late pregnancy to midlactation. The rate of synthesis of RNA in the preneoplastic hyperplastic alveolar nodule was unaffected by the transition of the host from the nulliparous to the pregnant state but was significantly decreased by the onset of lactation. The rate of DNA synthesis in the hyperplastic alveolar nodule was not significantly altered by these changes in the physiological state of the host. RNA synthesis in the neoplastic tissue was not significantly altered by changes in the physiological state of the host, but the rate of DNA synthesis in tumors from nulliparous mice was significantly lower than that in adenocarcinomas from lactating mice. To test whether the responses of the preneoplastic and neoplastic tissues were related to the hormonal changes in the host, nucleic acid synthesis in these tissues taken from mice that had been hypophysectomized five to seven days earlier were examined. Although the rate of RNA synthesis was unchanged in the tissues from the hypophysectomized mice, the rate of DNA synthesis was only 30% of that in comparable tissues from intact mice. The results of these studies are consistent with the concept that the preneoplastic and neoplastic mammary tissues of mice are sensitive to hormones. The levels circulating in the quiescent state are evidently sufficient to maintain nucleic acid synthesis so that changes due to pregnancy and lactation have no effect. When pituitary hormones are withdrawn by hypophysectomy, the dramatic decrease in the rate of DNA synthesis reveals the hormonal dependence of these abnormal mammary tissues.

6982 THREE CASES OF SUSPECTED MAMMARY NEOPLASIA IN NON-HUMAN PRIMATES. *(E.)* Appleby, E. C. (R. Vet. Coll., London, England), I. F. Keymer and J. M. Hime. *J Comp Pathol* 84(3):351-364, 1974.

Three cases of malignant neoplasia of the mammary region, two in *Galago crassicaudatus* and one in *Mandrillus sphinx*, are described and the histopathology illustrated. Case one was a malignant tumor, predominantly spindle-celled, which arose in the mammary region. It recurred after removal and metastasized to several sites. The secondary lesions in the lung, the liver and the kidney were mostly of a densely cellular, rather solid pattern, whose giant cells and other bizarre forms with a distinctive reticular network suggested a mesenchymal origin. Case two was a malignant tumor of mixed type with both acinar and spindle-cell components. which arose in the mammary gland. In the latest of the three biopsy specimens no glandular elements were seen, the cells appeared more plump and less fusiform, and there were areas resembling cartilage. The renal metastasis resembled the third biopsy specimen. In both cases silver preparations showed a distinct pattern of reticulin fibers in the metastatic foci. In case three, the lesion was of epithelial origin and was clearly an adenocarcinoma infiltrating the dermis and subcutaneous tissue of the thorax. The main body of the primary lesions may have been missed, but there is a strong presumption that this was adenocarcinoma of mammary origin. The possibility of a mixed tumor cannot be ruled out. Ten other reports of neoplasia of the mammary region in non-human primates are tabulated and briefly reviewed. With one exception, there appears to be no essential histological difference between these and various types of human breast cancer. In view of the importance of mammary neoplasia in man and the use of monkeys as experimental animals in cancer research, it is concluded that the mammary region of lower primates should always be carefully examined during necropsies for naturally occurring neoplasms. Details of all cases should be published.

6983 MALIGNANT OVARIAN TUMORS IN CHILDHOOD AND ADOLESCENCE. *(Fr.)* Paradis, D. (Chicoutimi Hosp., Canada) and L. Prive. *Tome* 103(9):1601-1607, 1974.

Five malignant ovarian tumors were removed from children and adolescents during the last ten yr. The tumors were located in the left ovary in four cases, and in the right ovary in one case. Histological examinations showed embryonic carcinoma, teratocarcinoma mixed with embryonic carcinoma, ovarian mesometanephroma and embryonic carcinoma with superimposed serous cystadenocarcinoma. A benign teratoma, which was independent of the primary tumor or possible recurrence occurred in one case. One patient died 5 months after surgery, and three are free from recurrences or metastases 18 to 51 months after surgery. The remaining patient, who is alive and well 73 months after surgery, had a pelvic tumor removed 64 months after the first operation. Although this might have been a recurrence, the tumor did not appear malignant. A mosaic chromosome pattern was found in one of three patients investigated, and α-fetoprotein was present in one of the two paitents with embryonic carcinoma; the α-fetoprotein disappeared a few days after surgery. Intrauterine irradiation in connenction with pelvimetry may be implicated in two cases, and extensive diagnostic irradiation 8 years before tumor diagnosis in another case.

6984 SPONTANEOUS REGRESSION OF DISSEMINATED NEUROBLASTOMA. *(E.)* Schwartz, A. D. (Child. Mem. Hosp., Chicago, Ill.), M. Dadash-Zadeh, H. Lee and J. J. Swaney. *J Pediatr* 85(6):760-763, 1974.

Two infants with disseminated neuroblastoma experienced spontaneous regression and recovery without the benefit of therapy. In a one-wk-old girl, the neuroblastoma involved the liver although the location of the primary tumor was undetected. The liver slowly decreased to normal size over the ensuing months; at one yr of age the child is well with no clinical, roentgenographic, or chemical evidence of tumor. In a two-month-old girl, the neuroblastoma involved the skin, the primary tumor probably arising from a left paravertebral sympathetic ganglion in the abdomen. The skin lesions completely resolved by 4 months of age. A review of the literature indicates a favorable prognosis for children with remote spread of tumor involving the liver, skin, and/or bone marrow without roentgenographic evidence of bone metastases (Stage IV S disease). Patients with remote metastases involving skeleton, internal organs, soft tissues, or distant lymph nodes (Stage IV disease) have the worst prognosis. Because of the complications of therapy, a conservative approach is recommended for treatment of children under one yr with Stage IV S disease. The incidence of spontaneous regression may be greater than is clinically evident.

6985 THE ASSOCIATION OF CONGENITAL MALFORMATIONS AND MALIGNANT TUMORS IN INFANTS AND CHILDREN. *(E.)* Valdes-Dapena, M. A. (St. Christopher's Hosp. Child., Philadelphia, Pa.), D. S. Huff and A. M. DiGeorge. *Ann Clin Lab Sci* 4(5):363-371, 1974.

Current information concerning the association between congenital anomalies and malignancies in pediatric patients is briefly reviewed. Such disorders have been classified into four groups: (1) Individuals with disorders characterized by chromosomal aberrations show a high incidence of: leukemia (Down's and Klinefelter's syndrome); testicular cancer, retinoblastoma, and Hodgkin's disease (Down's syndrome); Wilms' tumor and adrenal tumors (trisomy 13 and 18); and an unusual form of endometrial carcinoma in conjunction with estrogen treatment and neurogenic tumors (Turner's syndrome). (2) Patients with deficient immune responses have also been shown to be susceptible to cancer if they do not succumb to some infectious disease, e.g., lymphoreticular cancer (actasia-telangiectasia). (3) Genetic disorders, other than the three already mentioned, may be associated with familial clustering of malignancies, e.g., liver cancer; neuroblastoma in infants; the appearance of a second tumor of a different type in individuals with a primary tumor; and hereditary syndromes, where the increased incidence of malignancy occurs, e.g., familial polyposis. (4) Sporadic congenital developmental defects such as aniridia, hemihypertrophy and Beckwith's syndrome have been associated with Wilms' tumor, adrenal, and hepatic tumors. Four case histories are given.

6986 ISOLATION AND CHARACTERISTICS OF POLYADENYLIC ACID-RICH RNA FRACTIONS OF NORMAL AND TRANSFORMED RAT HEPATIC TISSUES. *(Rus.)* Ivanov, S. D. (N.N. Petrov Res. Inst. Oncol., USSR Minist. Health, Leningrad), J. P. Zerov, L. A. Voronova and I. F. Seitz. *Vopr Onkol* 20(6):84-87, 1974.

A method is described of preparative isolation of native highly-polymerized messenger RNA and heterogeneous nuclear RNA for the study of carcinogenesis. Diethyl nitrosamine-induced liver tumors in rats were used as models. Liver biopsies were performed at three stages of tumor growth: nonspecific carcinotoxic lesions (two weeks); benign transformations (2.5-3 months); and malignant transformations (six months). Poly(A)RNA was isolated by the method of Schutz *et al.* in which distilled water was replaced by diluted original eluent which helped obtain RNA practically free of polysaccharides. The isolated fractions were then studied by 2.5% polyacrylamide gel electrophoresis and poly(A) chromatography. The rate of incorporation of 5-^{3}H-uridine showed that poly(A)-rich fraction contained RNA with a fast rate of metabolism. The results indicate that in hepatocarcinogenesis in rats, the content of poly(A)-rich fraction in cytoplasmic RNA and nuclear RNA after two weeks of diethyl nitrosamine diet was 1.1% and 2.4%, resp; after 2.5 - 3 months, 0.78% and 3.3%; after six months, 1.4% and 3.3%, resp., (0.85% and 1.73% in the control). This shows that the quantity of the fraction in the cytoplasm did not change significantly in the course of carcinogenesis. It is suggested that some important changes in RNA metabolism occur in the cell nuclei during the stage of nonspecific carcinotoxic lesions.

6987 THROMBOCYTOSIS: PLATELET KINETICS IN NEOPLASIA. *(E.)* Tranum, B. L. (Univ. Arkansas Med. Cent., Little Rock) and A. Haut. *J Lab Clin Med* 84(5):615-619, 1974.

The survival time of autologous platelets, and the daily platelet turnover rate were measured using ^{51}Cr as a label in five patients who had thrombocytosis associated with neoplastic disease and a platelet count in excess of 550,000/μl. In all patients the life span of autologous platelets in the circulation was shorter than the normal value of 8.8 days (median value of 7.1 days). The disappearance of the labeled platelets was linear in all subjects without evidence of transient sequestration at the times selected for sampling. Although the rate of disappearance of the labeled platelets from the circulation varied in the subjects, in each case there was a high degree of correlation between the numbers of labeled platelets remaining in the circulation and the interval after their transfusion into the donor subject. The *in vivo* platelet recovery ranged from 24% to 86% (median value of 38%). In all five patients, the daily platelet turnover rate was increased about two- to four-fold greater than the calculated normal value. These data support the hypothesis that the phenomenon of thrombocytosis seen in association with metastatic neoplasia is due to an increased rate of platelet production, rather than to a prolongation of the platelet time in the circulation. In fact, the platelets have a mildly to moderately shortened *in vivo* survival as measured by *in vitro* labeling with ^{51}Cr. The increase in platelet production overcompensates for the reduction in platelet survival and can be construed to be a failure of normal feedback control mechanisms. It suggests that products of platelet destruction may stimulate or regulate the rate of platelet production.

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7067 ARGENTAFFIN CELLS IN THE NON-NEOPLASTIC MUCOSA, ADENOMA AND CARCINOMA OF THE STOMACH *(Jpn.)* Watanabe, H. (Fac. Med., Kyushu Univ., Fukuoka, Japan). *Gan No Rinsho* 20(7):519-535, 1974.

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7069 THE EVALUATION OF CATECHOLAMINE PATTERNS IN ELECTROMICROSCOPIC PICTURES IN CASES OF NEUROBLASTOMA IN CHILDREN. *(E.)* Borowicz, K. (Natl. Res. Inst. Mother and Child, Poland) and J. W. Borowicz. *Maandschr Kindergeneeskd* 42(11/12):454-455, 1974.

7070 ANAEMIA AND NEUROBLASTOMA IN CHILDREN. *(E.)* Tchernia, G. (Hosp. A. Beclere, Clamart, France). *Maandschr Kindergeneeskd* 42(11/12): 465-466, 1974.

7071 THE AGE OF THE PATIENTS WHO DIED OF STOMACH CANCER AND THE LOCALIZATION OF THE TUMOR. *(Pol.)* Duda, K. (Dept. Surg., Med. Acad., Krakow, Poland) and J. Kowalczyk. *Pol Przegl Chir* 46(11):1569-1574, 1974.

7072 ANGIOGRAPHIC STUDIES OF OSTEOBLASTOCIASTOMAS CERTAIN MORPHOLOGICAL CORRELATIONS. *(Rus.)* Mannanov, I. S. (Inst. Med. Radiol., Obninsk, USSR) and N. Iu. Polonskaia. *Ortop Travmatol Protez* (10): 29-33, 1974.

7073 HYPOXEMIA AND ACID-BASE BALANCE IN PATIENTS WITH ASBESTOSIS. *(Fr.)* Sessa, T. (Inst. Occupat. Med., Univ Naples, Italy), C. Vecchione, R. Mole and N. Javicoli. *Poumon Coeur* 30(2):135-139, 1974

7074 HISTOLOGICAL AND HISTOCHEMICAL FEATURES OF THE STROMA OF GASTRIC CARCINOMA. *(Rus.)* Zolotarevskii, V. B. (I. M. Sechenov 1st Moscow Med. Inst. USSR) and A. A. Vorobieva. *Arkh Patol* 36(11): 61-67, 1974.

7075 FURTHER KARYOLOGICAL EVIDENCE FOR CONTAGEOUSNESS AND COMMON ORIGIN OF CANINE VENEREAL TUMORS. *(E.)* Sasaki, M. (Chromosome Res. Unit, Fac. Sci., Hokkaido Univ., Sapporo, Japan), M. Oshimura, S. Makino, T. Koike, M. Itoh, F. Watanabe and N. Tanaka. *Proc Jpn Acad* 50(8):636-640, 1974.

7076 ULTRASTRUCTURAL STUDY OF ENDOMETRIAL STROMAL SARCOMA OF STROMAL ENDOMETRIOSIS. Kudo, R. (Sapporo Med. Coll., Japan), A. Komori and M. Hashimoto. *Acta Obstet Gynaecol Jpn* 20(2):72-78, 1973.

See also:

* (Rev): 6607, 6612, 6642, 6643, 6644, 6645, 6646, 6647, 6648, 6649, 6654, 6655, 6659, 6678, 6680, 6681, 6682
* (Chem): 6690, 6698, 6726, 6733, 6756
* (Phys): 6773, 6780
* (Viral): 6795
* (Immun): 6864, 6960
* (Epid-Biom): 7078, 7079, 7099

7077 CARCINOGENIC AIR POLLUTANTS IN THE EXHAUST FROM A EUROPEAN CAR OPERATING ON VARIOUS FUELS.

(E.) Candeli, A. (Inst. Hygiene, Perugia, Italy), V. Mastrandrea, G. Morozzi and S. Toccaceli. *Atmos Environ* 8(7):693-705, 1974.

The influence of tetraethyl lead (TEL) content (0 and 0.63 g/liter), fuel aromaticity (0, 6, and 48%), and type of polycyclic aromatic hydrocarbon at a constant total aromatic level (benzene, xylene, and emission of polycyclic aromatic hydrocarbons (PAH) from a typical European car engine (1608 cm^3) without emission control was studied. Various fuels were used, with each test consisting of European test cycles on a dynamometer bench. In two cases, the addition of TEL reduced the PAH emission, while in a third case it increased the emission level, indicating that the effect of TEL and PAH is not generalizable. Increasing fuel aromaticity increased PAH emission; however, these results applied only to the three leaded test fuels and not to unleaded versions of the same fuels. With three synthetic unleaded fuels, the type of aromatic compound present strongly affected the PAH emission levels. Simple aromatic hydrocarbons, such as benzene and xylene, produced less PAH than the C_9 and C_{10} hydrocarbons.

7078 WHAT IS BURKITT'S LYMPHOMA?

(E.) Morrow, R. H., Jr. (Harvard Sch. Public Health, Boston, Mass.), P. H. Levine, J. L. Zeigler and C. Berard. *Lancet* 2(7891):1268-1269, 1974.

Reports of the lack of association between Epstein-Barr virus and Burkitt's lymphoma in the United States bring out the question of what constitutes Burkitt's lymphoma. In comparison with African patients, American patients with Burkitt's lymphoma tend to be older, to present with advanced intraabdominal tumors, and to have atypical manifestations. The prolonged response to chemotherapy that is characteristic of most Burkitt's lymphoma patients in Africa is seen less frequently. Thus, caution is indicated in the interpretation of even those cases that meet the histopathological criteria outlined by the World Health Organization. However, the American Burkitt Lymphoma Registry has now identified 141 cases of Burkitt's lymphoma, and the clinical and laboratory features continue to show notable similarities between African and American Burkitt's lymphoma. The more rigorous the histopathological and cytological criteria used, the more striking are the similarities in the associated clinical features.

7079 SINUS HISTIOCYTOSIS IN BRITISH AND JAPANESE PATIENTS WITH BREAST CANCER.

(E.) Friedell, G. H. (St. Vincent Hosp., Worcester, Mass.), E. A. Soto, S. Kumaoka, O. Abe, J. L. Hayward and R. D. Bulbrook. *Lancet* 2(7891):1228-1229, 1974.

Sections of both primary tumor and regional lymph nodes from 86 Japanese and 86 British patients with breast cancer were reviewed "blind" by two American pathologists. Sinus histiocytosis was present in 57% of the Japanese series but in only 4% of the British, and this difference persisted when axillary-node metastasis was taken into account. For patients with axillary-node metastases, the frequency of sinus histiocytosis was 39% of 31 cases in the Japanese series and 3% of 26 cases in the British. In the absence of axillary-node metastases, sinus histiocytosis occurred in 67% of 55 Japanese and 11% of 50 British cases. If histiocytosis represents an immunological defense mechanism, (as previously suggested) these results may explain the different incidence of invasive cancer in the two populations studied.

7080 A SURVEY OF THE USE OF OESTROGENS DURING PREGNANCY IN THE UNITED KINGDOM AND OF THE GENITO-URINARY CANCER MORTALITY AND INCIDENCE RATES IN YOUNG PEOPLE IN ENGLAND AND WALES.

(E.) Kinlen, L. J. (Radcliffe Infirm., Oxford, England), M. A. Badaracco, J. Moffitt and M. P. Vessey. *J Obstet Gynaecol Br Commonw* 81(11):849-855, 1974.

To obtain some estimate of the frequency of estrogen therapy during pregnancy in the United Kingdom, questionnaires were sent to 659 Fellows of the Royal College of Obstetricians and Gynaecologists. From information provided by 544 respondents, it was estimated that about 7500 women were treated with diethylstilbestrol during pregnancy from 1940-1971, the majority during the 1950's. Other estrogens were given to approximately a further 4500 patients, mainly during the 1960's. Analysis of genitourinary cancer mortality and incidence rates in men and women under 30 yr in England and Wales showed a rise in the incidence of cancer of the cervix and a rise in both the incidence of mortality from cancer of the testis. However, there is little reason to suspect a connection between these trends and *in utero* exposure to estrogens. An increased incidence of cervical cancer was also apparent in women aged 30-44 yr, and rising mortality from cancer of the testis in young men was already evident before the late 1940's, when estrogens were first introduced into obstetric practice. Although persuasive evidence of an association between adenocarcinoma of the vagina or cervix and *in utero* exposure to diethylstilbestrol has come from the United States, no case of genital tract adenocarcinoma occurring in a similarly exposed young woman has yet been reported in the United Kingdom. Since the present study indicates that the number of women so exposed is considerable, it is possible that such tumors will begin to appear within the next few years.

7081 A PROSPECTIVE STUDY IN GENERAL PRACTICE ON BREAST-CANCER RISK IN POSTMENOPAUSAL WOMEN.

(E.) De Waard, F. (Inst. Soc. Med., Univ. Utrecht, Netherlands) and E. A. Baanders-van Halewijn. *Int J Cancer* 14(2):153-160, 1974.

A prospective study of postmenopausal women was undertaken to investigate possible relationships between nutritional status, hormonal status, and breast-cancer risk. Fifty general practitioners interviewed and examined 7,259 women, then followed the cases for an average of 5.4 yr; 70 cases of breast cancer were observed. The relative breast-cancer risk increased with increasing weight of the patient. Height also proved to be a risk factor. Cases of both diabetes and hypertension had only slight increases in risk but were often associated with high body weight. Women with a history of unilateral mastectomy had a calculated risk of 5.6 times normal. Single women did not show high risk but tended to have lower body weight. Multiparity was shown to have some protective effect in heavy women, but the study was done before the protective effect of early pregnancy was demonstrated. The evidence suggests that height and weight are synergistic in their effect on breast-cancer risk, but total body size rather than weight per unit of height determines risk. This risk, attributable to weight and height may be of practical importance in explaining a substantial part of international variations in breast cancer incidence.

7082 STATISTICAL CONSIDERATION OF URINARY TRACT TUMORS FOUND IN THE LAST TWENTY YEARS AT THE UROLOGY DEPARTMENT, VERCELLI HOSPITAL. *(It.)*

de Benedetti, H. (S. Andrea Hosp., Vercelli, Italy), P. G. Conti and E. Barasolo. *Minerva Urol* 26(1):21-24, 1974.

Statistics were assembled on the occurrence of genito-urinary tract tumors over a 20 year period. From an examination of raw figures, an increase in bladder tumors (papillomatous and non-papillomatous) and in prostate tumors was noted. Since no follow-up on benign bladder tumors was possible, connections between the papillomatous and non-papillomatous forms in the cases studied could not be determined.

7083 NASOPHARYNGEAL CARCINOMA AT THE TEL-HASHOMER HOSPITAL, ISRAEL: A 20 YEAR SURVEY (1951-1970). *(E.)*

Har-Kedar, I. (Tel-Hashomer Hosp., Israel), S. Chaitchik and A. Hercberg. *Clin Radiol* 25(3):403-407, 1974.

A clinical survey is presented of 89 cases of carcinoma of the nasopharynx seen at the Sheba Medical Centre in Israel from 1951-1970. The series included 68 men and 21 women; 25% of the patients were under the age of 30. The ethnic distribution was 54% Sephardim Jews, 20% Ashkenazim, and 26% Arabs; the percentages of Sephardim and Arabs were higher than expected. The predominant histological type was lymphoepithelioma, and its incidence increased over the past five yr to 67% of all the findings (49 cases). The other types were reticulum cell sarcoma (7), squamous cell carcinoma (17), anaplastic carcinoma (14), and unidentified carcinoma (6). In 22% of the case the tumors were localized and in the remaining cases there were already signs of dissemination. Six of nine patients under age 14 presented with bone metastases compared with only six of 80 older cases. A third of the patients died within the first year after diagnosis; approximately 27% were alive three yr after therapy; and the five-yr survival rate was less than 10%. The overall poor survival rate may be a result of the conservative nonaggressive treatment employed up to 1969. Since the adoption in 1969 of the technique reported by Lederman, the survival rate has improved 40% over three yr.

7084 LUNG CARCINOMA: DETECTION OF RISK-COLLECTIVES AND EARLY SYMPTOMS. *(E.)*

Trendelenburg, F. (Dep. Pulm. Dis., Univ. Hosp., Homburg, Germany). *Scand J Respir Dis Suppl* 89:79-83, 1974.

The early symptoms of lung carcinoma and their application to the prediction of patients at risk of developing this disease are discussed. Even at the early detection stage, only about 50% of affected patients are operable; and of the operable patients, a maximum of 80% have a life expectancy of five yr at this stage. During later stages of the disease, the life expectancy falls off rapidly. A questionnaire has been developed which includes questions concerned with initial symptoms and which can be applied to the definition of persons who are at risk of bronchial carcinoma. The questions relate to the patients age and sex, smoking habits, previous lung disorders, and experiences with chest pains, coughing, and the coughing up of phlegm and blood. The predictive value of these questions ranges from 69-83% in the case of positive answers and from 35-54% in the case of negative answers. This special questionnaire is suitable for use in practice in outpatient clinics and hospitals, and in occupational medicine.

7085 CELL KINETICS IN BURKITT LYMPHOMA. *(E.)*

Iversen, O. H. (Inst. Pathol., Univ. Oslo, Norway), U. Iversen, J. L. Ziegler and A. Z. Bluming. *Eur J Cancer* 10(3):155-163, 1974.

The cell kinetics of small pieces of diagnostic biopsies of African cases of Burkitt lymphoma were studied in 13 cases of primary tumor, four cases of recurring tumor and three cases of non-Burkitt lymphosarcomas in short time tissue culture with tritiated thymidine (3HTdr) in the medium. The *in vivo* uptake of 3HTdr was also studied in two patients in Stage IV of the disease. The uptake of 3HTdr by tumor cells in ascites fluid was studied in one patient. On seven patients who received 2-4 hr prebiopsy Colcemid the number of Colcemid-arrested mitoses were counted. The actual growth rate of four cutaneous nodules of Burkitt tumor was measured. The results were analyzed by means of a mathematical tumor growth model and the kinetic parameters tabulated. The kinetic parameters observed were as follows: actual clinical doubling time, 66 hr; labeling index, 17.34%; rate of cells entering DNA-synthesis, 2.71%/hr; mitotic index, 1.27%; rate of cells entering mitosis, 1.75%/hr; duration of G_1, 16.5 hr; duration of DNA-synthesis, 6.5 hr; duration of G_2, 2 hr; duration of mitosis, 0.7 hr; potential doubling time based on labeling indices, 25.6 hr; potential doubling time based on number of Colcemid arrested mitosis, 39.6 hr; cell loss in the G_2 phase, 30%; and total cell loss factor, 69% of maximum possible cell gain. When the kinetic parameters of each of these tumors were compared to the clinical data, no useful correlations were observed. It is concluded that cell proliferation in Burkitt tumor is very rapid, but there are great differences in the rate of cell renewal between cases. These variations are not dependent on the age of the patient, whether the tumor was primary or a recurrence, or the location of the tumor.

7086 OCCUPATIONAL AND NON-OCCUPATIONAL SCROTAL CANCER IN SWEDEN, 1958-1970. *(E.)* Wahlberg, J. E. (Karolinska Hosp., Stockholm, Sweden). *Acta Derm Venereol (Stockh)* 54(6):471-474, 1974.

Between 1958-1970, the Swedish Cancer Registry received reports of 34 cases of scrotal squamous cell carcinoma. All were histopathologically verified. The age at onset was 45-85 yr, with the highest incidence between 60-69 yr. Seven patients (21%) had a long history (15-39 yr) of exposure to oil as automatic lathe operators. Six patients (18%) had a history of some hand contact with motor oils, but they had not been subjected to the soaking of clothes experienced by automatic lathe operators. Among 21 patients (62%) with no contact with oil, there were three cases of multiple Mb Bowen, basalioma, and squamous cell carcinoma; one case of internal multiple cancers; four with concurrent psoriasis; and four severe psychiatric cases. The majority of cases (80%) were of nonoccupational origin. Of the 16 patients who died during the observation period (minimum of four yr), only one had lengthy exposure to oil; this subject (aged 74 yr) had developed a primary lung cancer (oat-cell), which could be related to exposure to oil mist. One patient with some exposure to oil also died from oat-cell cancer in the lungs, and primary bronchial cancer (squamous cell carcinoma) was the cause of death in a patient with no contact with oil. The smoking habits of these subjects are not known.

7087 TURNOVER OF CELL-RENEWING POPULATIONS UNDERGOING CIRCADIAN RHYTHMS IN CELL PROLIFERATION. *(E.)* Izquierdo, J. N. (Sch. Med. Dent., Univ. Rochester, N. Y.) and S. J. Gibbs. *Cell Tissue Kinet* 7(2):99-111, 1974.

The cytokinetics of cheek pouch squamous epithelia (known to undergo circadian mitotic and DNA-synthetic rhythms) in 204 Syrian golden and 16 Chinese hamsters were investigated to determine turnover times and whether or not there exists a non-proliferating G_0 subpopulation. The animals received repeated injections of ^{3}H-thymidine. Mitotic and labeling indices and percent labeled mitoses from Syrian hamsters were scored for each animal. In the Syrian hamster cheek pouch epithelium, the mitotic indices and labeling indices showed marked circadian rhythms. Age distribution of cells in S, G_2 and M phases were constructed using mitotic indices. The age-distribution curves showed that cells were not uniformly distributed throughout the cycle. The age-distribution function was a complex one which changed with the time of day. This indicates that equations based on total asynchrony are not applicable for determination of proliferation rate and turnover time. An approximately linear increase in the labeling index following repeated injections of ^{3}H-thymidine demonstrated that nearly 100% basal cells in hamster cheek cells were in cycle during a turnover time. These experiments suggest that if there is a G_0 phase in cell renewing tissues, this is short with respect to turnover time and that it may be a specific compartment where the control of cell proliferation operates.

7088 CELL KINETICS OF HUMAN GLIOMAS. *(E.)* Hoshino, T. (Univ. California Sch. Med., San Francisco) and C. B. Wilson. *Recent Prog Neurol Surg, Proc Symp Int Congr Neurol Surg, 5th, Tokyo, Oct 7-13, 1973 (Amsterdam: Excerpta Medica)* pp. 12 128, 1974.

To determine the parameters of brain tumor growth (ce cycle or generation time, growth fraction, cell loss factor, and tumor doubling time) double radioautograp techniques and stathmokinetic methods were used. In malignant gliomas, the labeling index (ratio of numbe of labeled cells in the DNA synthesis to total tumor cell population) varied from one place in the tumor to another, ranging from 20% in the most viable area of the tumor to almost nothing in necrotic areas. Th range is attributable to the heterogenicity of the tu itself. S phase duration in the mitotic cycle was es timated in 9 brain tumors by means of double radioautography. With two exceptions, it remained in the 7-10 hr range regardless of tumor type and degree of malignancy. Tumor type dependency and degree of malignancy can be excluded as factors affecting S phase duration *in vivo*. A rough estimation of the cell loss factor in glioblastomas, assuming a potenti doubling time of 1 week and a clinical doubling time of 5-6 weeks, gives a cell loss factor of 0.8-0.85. This means 80-85% of newly-added tumor cells are lost (value may vary from time to time or patient to patie Patients with recurrent glioblastomas received an intracarotid injection of ^{3}H-thymidine during the cou of selective angiography approximately one week befor operation. A mitostatic agent, vinblastine sulfate, was given 4-6 hr before operation, not only to increase the number of cells in mitosis, but also for stathmokinetic analysis of cell cycle time. Specimen of tumor removed at operation were prepared by standa autoradiographic methods, and the numbers of labeled cells in mitoses were computed. The growth fraction of glioblastomas is 0.3-0.4 and cell cycle time of proliferating cells is in the order of 2-3 days. Based on the present knowledge of the kinetic parameters of human glioblastomas the author suggests tha a single course of antimetabolites and other cell-cyc specific agents against this tumor would be ineffecti

7089 EVOLUTION OF MORTALITY RATES BY CANCER OF THE COLON IN SPAIN. *(Sp.)* Palmeiro Troitiño, R. (Inst. Natl. Oncol., Madrid, Spain) and A. Senra Varela. *Rev Sanid Hig Publica (Madr)* 47(6): 517-528, 1973.

7090 CHANGES OF MORBIDITY AND THERAPY OF MALIGNANT MELANOMA ON THE DEPARTMENT OF DERMATOLOGY OF THE MEDICAL UNIVERSITY IN DEBRECEN. *(Hung.* Torok, E. (Clin. Dermatol., Med. Univ., Debrecen, Hungary). *Mag Onkol* 18(2):78-81, 1974.

7091 EXPERIMENTAL STUDY OF THE ETIOPATHOLOGICAL ROLE OF EROSIVE SILICATES IN THE PATHOGENES OF ENDEMIC NEPHROPATHY AND OF TUMORS OF THE URINARY TRACT. *(Fr.)* Markovic, B. (Med. Cent. Gnjilane, Gnjilane, Yugoslavia). *Seante Publique (Bucur)* 17(4) 467-473, 1974.

7092 THE EPIDEMIOLOGIC SITUATION OF BRONCHIAL AND PULMONARY CANRINOMA IN THE POPULATION FROM THE TERRITORY OF ZAGREB. *(Ser.)* Radosevic, Z. (Inst. Tuber. Lung Dis., Zagreb, Yugoslavia). *Plucne Bolesti Tuberk* 26(Suppl. 2):264-269, 1974.

7093 EPIDEMIOLOGICAL FEATURES OF CANCER IN IRAN. *(Fr.)* Habibi, M. A. (Fac. Med., Teheran, Iran). *Bull Acad Natl Med (Paris)* 158(8):636-641, 1974.

7094 PROSTATE CANCER IN ÖREBRO COUNTY FROM 1958-1967. *(Sw.)* Dolk, T. (Regional Hosp., Örebro, Sweden) and A. Fritjofsson. *Lakartidningen* 71(38):3517-3519, 1974.

7095 LUNG CANCER MORTALITY AT LKAB'S MINES AT MALMBERGET. *(Sw.)* Goran St. Clair Renard, K. (LKAB Mines, Malmberget, Sweden). *Lakartidningen* 71(3):158-162, 1974.

7096 THE INCORPORATION OF RADIOACTIVE THYMIDINE FOR MEASUREMENT OF THE RATE OF TUMOUR GROWTH. *(Ger.)* Wayss, K. (Inst. Exp. Pathol., Hedelberg, West Germany), J. Mattern, M. Volm and J. Zimmerer. *Arch Geschwulstforsch* 44(4):307-311, 1974.

7097 TUMORS OF THE URINARY TRACT AND OF THE BRAIN IN RUBBER WORKERS. *(Ger.)* Lamperth-Seiler, E. (Blumenfeld 17, CH-6460, Altdorf, Switzerland). *Schweiz Med Wochenschr* 104(46):1655-1659, 1974.

7098 REANALYSIS OF THE DATA CONCERNING CANCER OF THE LUNG IN FREDERICIA. *(Dan.)* Andersen, E. (Stat. Inst., Copenhagen, Denmark). *Ugeskr aeger* 136(48):2704-2705, 1974.

7099 THE NATURAL HISTORY OF NEUROBLASTOMA: A STUDY OF 487 CASES. *(E.)* Wilson, L. M. . (Marie Curie Mem. Found., The Chart, OXTED England). *Maandschr Kindergeneeskd* 42(11/12):467-468, 1974.

7100 MYELOPROLIFERATIVE SYNDROME, MYELOFIBROSIS AND ERYTHROCYTOSIS. A RETROSPECTIVE STUDY. *(Afr.)* Retief, F. P. (Dept. Internal Med., Orange Free State Univ., Bloemfontein, South Africa) and A. du P. Heyns. *S Afr Med J* 48(59):2429-2435, 1974.

7101 MORTALITY FROM CANCER OF THE BUCCAL CAVITY AND PHARYNX. *(Pol.)* Staszewski, J. (Inst. Oncol., Gliwice, Poland). *Nowotwory* 24(3):167-172, 1974.

7102 BRONCHIAL CANCER IN BERLIN, CAPITAL OF THE G.D.R. 1ST COMMUNICATION: SURVIVAL RATES IN 1960-1964 AND IN 1965. *(Ger.)* Lehmann, C. (Dept. Public Health and Welfare, Magistrate of Great Berlin, East Germany) and H. Berndt. *Arch Geschwulstforsch* 44(4):371-381, 1974.

7103 OBSERVATIONS ON THE DISEASE AND DEATHS CAUSED BY THE CARCINOMA OF THE RESPIRATORY SYSTEM ON THE TERRITORY OF THE COMMUNE CENTARSARAJEVO IN THE PERIOD 1960-1970. *(Ser.)* Laketić, S. (Health Center, Sarajevo, Yugoslavia), D. Banjac, B. Trninić, F. Metz, N. Jovanović, S. Stevanović and F. Kulenović. *Plunce Bolesti Tuberk* 26(Suppl. 2):387-389, 1974.

7104 A NEW LOOK AT THE BIOLOGY OF HUMAN CANCER. *(E.)* Metcalf, D. (Melbourne, Australia). *UICC Bull* 12(4):4, 1974.

See also:

- * (Rev): 6612, 6650, 6651, 6661, 6662
- * (Immun): 6870
- * (Path): 6980, 7035, 7044, 7052, 7062, 7064, 7071, 7073

7105 HEMOGLOBIN-SYNTHESIZING MOUSE AND HUMAN ERYTHROLEUKEMIC CELL LINES AS MODEL SYSTEMS FOR STUDY OF DIFFERENTIATION AND CONTROL OF GENE EXPRESSION. *(E.)* Dube, S. K. (Max-Planck Inst. Exp. Med., Gottingen, West Germany), G. Gaedicke, N. Kluge, B. J. Weimann, H. Melderis, G. Steinheider, T. Crozier, H. Beckmann and W. Ostertag. *Proc Int Symp Princess Takamatsu Cancer Res Fund, 4th,* 88-132, 1973.

Induction of hemoglobin synthesis was studied in cell lines and clones established from Friend virus-transformed mouse erythroleukemic cells and in human cell lines established from patients with polycythemia vera and erythroleukemia. In the uninduced state, little hemoglobin was synthesized by mouse spleen, liver, or peripheral blood cells *in vitro*. Addition of dimethyl sulfoxide (DMSO, 1-2%), cyclic AMP (10^{-4} M), or etiocholanolone (10^{-7} M) to the culture medium markedly stimulated erythroid differentiation in these cells. Under favorable conditions, the cells were able to synthesize up to 50% hemoglobin composed of α and β chains. A decrease in membrane permeability after the addition of DMSO, etiocholanolone, or cyclic AMP was followed by a decrease in nuclear RNA synthesis and then a decrease in label in cytoplasmic RNA. Synthesis of globin mRNA was augmented, as shown by the appearance of newly labeled globin mRNA in the cytoplasm. Induced globin synthesis peaked on day five, when DNA synthesis and cell division stopped. The increase in available globin mRNA after induction may be attributable to increased transcription; furthermore, there appears also a control at the level of translation. Most of the globin template activity in five-day induced cells was in the polysomal fraction, while in uninduced cells the globin template activity was mostly in the monosomal fraction. Induction with DMSO was not possible with bone marrow cells from polycythemia vera or erythroleukemia patients. However, carboxymethylcellulose-urea column elution patterns of the cell lines indicated that the cells synthesized small amounts of globin chains; more β than α chains.

7106 SELF-REGULATION OF GROWTH IN THREE DIMENSIONS: THE ROLE OF SURFACE AREA LIMITATION. *(E.)* Folkman, J. (Child. Hosp. Med. Cent., Boston, Mass.), M. Hochberg and D. Knighton. *Cold Spring Harbor Conf Cell Proliferation* 1:833-842, 1974.

The self-regulation of three-dimensional growth of cultured tumor cell aggregates was studied in spheroids removed from B-16 melanoma, V-79 Chinese hamster, and L-5178Y leukemia cells suspended in soft agar. After 5-23 weeks of linear growth in fresh agar, all spheroids reached a critical diameter beyond which no further expansion occurred (stage of population dormancy). Spheroids of 0.3 mm diameter had a central necrotic zone, a middle zone of unlabeled cells, and an outer zone of ^{3}H-thymidine-labeled cells with accompanying mitoses. From 5-7 outer layers were labeled in spheroids up to 1.0 mm diameter. As the spheroids enlarged further toward the dormant state, the ratio of volume of labeled cells to the total volume of the spheroid decreased until in the dormant phase the labeled cells occupied only a single outer layer of the spheroid. No amount of nutrient change would make a spheroid larger. In all spheroids a maximum cell number was reached long before the diameter stopped expanding; further expansion was due to the accumulating necrotic center. *In vivo* studies of tumors (e.g., Walker 256 carcinoma, B-16 melanoma, mouse adenocarcinoma) implanted in the chorioallantoic membrane of chick embryos revealed that tumors remained dormant during the refractory state of the chorioallantoic blood vessels. If tumor angiogenesis factor (a mitogen specific for endothelial cells of capillaries and small veins) could be blocked, then solid tumors might be held in the avascular or first stage of tumor growth.

7107 EFFECTS OF PROTEASE INHIBITORS ON GROWTH OF 3T3 AND SV3T3 CELLS. *(E.)* Chou, I. N. (Harvard Med. Sch., Boston, Mass.), P. H. Black and R. Roblin. *Cold Spring Harbor Conf Cell Proliferation* 1:339-350, 1974.

Experiments were performed to determine the effects of the chymotrypsin inhibitor *N*-tosyl-L-phenylalanyl-chloromethylketone (TPCK) and the trypsin inhibitor *N*-tosyl-L-lysyl-chloromethylketone (TLCK) on the growth of Simian virus 40 (SV40)-transformed Swiss and BALB/c 3T3 cells. Growth of Swiss SV3T3 cells was unaffected by TPCK at concentrations below 100 µg/ml. At 150 µg/ml TPCK caused a marked decrease in growth rate and at 200 µg/ml such severe growth inhibition occurred that cells stopped increasing in total cell number. This resulted in the appearance of a growth plateau approximating the saturating density of contact-inhibited untransformed 3T3 cells. BALB SV3T3 cells were somewhat less sensitive to TPCK inhibition. The TPCK-mediated growth inhibition was reversible, could be partially overcome by serum and was not selective against transformed cells since growth of untransformed cells was similarly affected. Doses of TPCK required to stop cell growth also reversibly inhibited both protein and DNA synthesis. Cycloheximide (0.5-0.6 µg), a protein synthesis inhibitor, caused a plateau phenomenon which mimicked that induced by TPCK. TLCK was not inhibitory to Swiss or BALB/c SV3T3 cells at 100 µg/ml and was only slightly inhibitory at 150 µg/ml. At 200 µg/ml TLCK was toxic for both transformed and untransformed 3T3 cells. These results strongly suggest that the growth inhibition observed in the presence of TPCK is a consequence of overall inhibition of protein synthesis, rather than the selective inhibition of a protease-like activity.

7108 THE MECHANISM OF THE AUTONOMY OF DIVISION OF ASCITIC TUMOR CELLS. *(E.)* Malenkov, A. G. (Inst. Biol. Test. Chem. Compd., Moscow, USSR), V. A. Artamonova, M. L. Kakushkina, G. G. Slivinsky and V. L. Voejkov. *Int J Cancer* 14(5): 691-697, 1974.

A bovine serum gradient was used to separate several strains of ascites tumor cells into fractions according to size and position in the cell cycle.

lls from different fractions were treated with
ase or tween 80, and the influence of this treat-
nt on protein synthesis, membrane permeability,
d the dry mass of the cells was studied. Both
ase and tween 80 stimulated protein synthesis
d increased the dry mass only in cells with a
all initial mass and size. The distribution of
-thymidine incorporation into the cells suggested
at the cells with a small size and/or mass were
the beginning of the mitotic cycle. In larger
lls which appeared later in the cell cycle, RNase
used either no stimulation or a slight inhibition.
e addition of actinomycin D to the incubation
dium completely prevented the RNase-stimulated
crease in the dry mass and protein synthesis of
e smaller cells. The concentrations of tween 80
ich resulted in maximum stimulation of protein
nthesis caused a slight increase in the ion per-
ability of the cell surface, and the effect of
ase on cell metabolism appeared to be due entirely
changes in the plasma membrane. The results
ggest that the increase in plasma membrane perme-
ility caused by RNase and tween 80 may initiate
A and protein synthesis in ascitic tumor cells
the G_1 phase. Thus, permeability increase in the
phase is the probable cause of the initiation of
vision in ascites tumor cells.

09 THE EFFECT OF CHALONE ON THE CELL CYCLE IN THE EPIDERMIS DURING WOUND HEALING. *(E.)*

Ymaguchi, T. (Natl. Inst. Radiol. Sci., Anagawa,
Clba, Japan), T. Hirobe, Y. Kinjo and K. Manaka.
Cell Res 89(2):247-254, 1974.

effect of epidermal chalone on the mitotic and
synthetic activities in regenerating epidermis
ter a cut was studied using 10-12 month old male
tley albino guinea pigs, or 4-6 month old male
BL/6J mice to obtain the epidermal chalone. A
was made on the ear conch of some C57BL/6J mice
l two days later an extract containing epidermal
lone was injected s.c. The time changes after
lone treatment in the number of cells labeled with
^{3}-thymidine, in the number of grains on labeled cells,
l in the number of mitoses within the regenerating
dermis surrounding the wound were investigated by
oradiography (ARG). The autoradiographs were ex-
sed at 4 C and developed after an exposure of either
or six weeks (short and long exposure, resp.).
in counts decreased temporarily in early phase
2 hr) after chalone injection; this resulted in a
crease in the number of labeled cells on the ARG of
short exposure but not of a long exposure. A de-
ase in the number of labeled cells on the long
osure was evident at 6 hr when the grain counts
erted to a level similar to the controls without
lone. Mitoses reached a minimum at two hr and
n increased, indicating a rapid disappearance of
inhibition of cells in G2 from entering M phase.
oses decreased thereafter, indicating inhibition
cells in the preceding S phase from completing DNA
thesis. An extract made from liver or kidney tissue
not affect the mitotic or DNA synthetic activities.
results indicate that the epidermal chalone in-
ited the epidermal cell proliferation.

7110 THE ROLE OF CRITICAL CELL PROLIFERATION IN DIFFERENTIATION OF MAMMARY EPITHELIAL CELLS.

(E.) Topper, Y. J. (Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, Md.) and B. K. Vonderhaar. *Cold Spring Harbor Conf Cell Proliferation* 1:843-852, 1974.

Cell proliferation in mammary gland epithelial cells of C3H/HeN mice was studied using agents that inhibit DNA synthesis, i.e., arrest the cells in the late G_1 phase. The epithelial DNA content in explants from mature virgin mammary glands increased in the presence of insulin, hydrocortisone, and prolactin. This increment was blocked when either 1-β-D-arabinosylcytosine (ara-C) or fluorodeoxyuridine (FdU) was added at zero time. In the absence of these inhibitors, a large increase in casein synthesis occurred after 72 hr, while essentially no increase took place in their presence. Addition of insulin, hydrocortisone, and prolactin at zero time prevented the emergence of B-protein (α-lactalbumin) activity in explants from mature virgin mice. However, addition of ara-C or FdU prior to emergence of B-protein but after initiation of DNA synthesis did not depress the B-protein. Neither ara-C nor FdU blocked the increased ratio of glucose-6-phosphate dehydrogenase + gluconate-6-phosphate dehydrogenase:DNA elicited by insulin, hydrocortisone, and prolactin. Studies on tissue from mature virgin mice primed with estradiol showed that insulin and serum promoted critical mitosis. In explants from mice in mid-pregnancy, ara-C inhibited DNA synthesis and lowered both casein and B protein production. However, synthesis of these proteins per cell was virtually unaffected by the nucleoside. Pregnancy tissue differed from virgin tissue in this respect. Epithelial cells in involuted mouse mammary glands were devoid of B-protein activity but cells in the mid-pregnant mouse could make this secretory protein in response to insulin, hydrocortisone, and prolactin. These results indicate that cell proliferation is a prerequisite for formation of secretory proteins only in tissue from virgin mice.

7111 CHARACTERIZATION OF DIFFERENTIATED AND DEDIFFERENTIATED CLONES FROM A RAT HEPATOMA.

(E.) Deschatrette, J. (Cent. Mol. Genet. CNRS, Gif-sur-Yvette, France) and M. C. Weiss. *Biochimie* 56(11/12):1603-1611, 1974.

The spontaneous dedifferentiated variants isolated from the Reuber H35 rat hepatoma-derived line H4HEC3 were characterized with respect to the production of serum albumin and the activity of four liver-specific enzymes. The majority of these clones expressed in a stable fashion in a number of liver specific functions,,including secretion of serum albumin, alcohol dehydrogenase and aldolase activity, and a high basal activity and hormone inducibility of tyrosine aminotransferase and alanine aminotransferase. Although the differences in the level of expression of these functions extended over a range of 5-10-fold, the variations were not consistent within or between clones. Seven clones which differed from most of the others in terms of morphology and the expression of liver-specific functions were isolated. In five of them, there was no detectable expression of any of the liver functions, while two of them showed diminished but significant expression of two or three of the functions. In all clones studied there was a negative correlation between glucose-6-phosphate dehydrogenase (G6PD) activity and the expression of liver-specific functions; the expression of this enzyme was not modified by treatment with 5-bromodeoxyuridine or N^6, O$^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphate or by growth as tumors in Buffalo rats. The results suggest that the presence or absence of G6PD is most likely due to some kind of regulatory phenomenon; this phenomenon may be associated with the neoplastic state or cell culture.

7112 ENERGY METABOLISM OF BURKITT LYMPHOMA CELLS.

(E.) Terranova, T. (Inst. Gen. Pathol., Cathol. Univ., Rome, Italy), A. Dani, A. Cittadini, G. Longhi, D. Bossi, G. M. Bartoli, C. Chezzi and M. Russo. *Proc Fifth Int Symp Biol Characterization Hum Tumours, Bologna, April 4-6, 1973* 1:85-91, 1974.

The respiratory activities of Burkitt lymphoma cells and of normal rat thymus cells under a variety of incubation conditions were compared. Burkitt lymphoma cells were grown in Joklik's medium and harvested 7-10 days after inoculation. The oxygen consumption of isolated Wistar rat thymus cells was measured manometrically during 60 min incubation at 38 C in the presence of 10 mM of glucose. The lactate was measured in the neutralized perchloric acid extracts by an enzymic method; lactate dehydrogenase activity was determined in cell-free extracts by following the rate of NADH oxidation at 366 nm in the presence of various concentrations of pyruvate. Pyridine nucleotide kinetics were carried out at 340-375 nm at room temperature. The effect of a variety of respiratory inhibitors (oxamate, 2,4-dinitrophenol, oligomycin, iodacetate, amino-oxyacetic acid and sodium fluoride) was measured. Under these various incubation conditions Burkitt lymphoma cells manifested a weak Pasteur effect (very small increase in lactic acid production on switching to anaerobic conditions) and a very marked Crabtree effect (sharp decrease in O_2 consumption when switching from < 1 mM glucose to 20 mM glucose). It is concluded that Burkitt cells like many other tumor cells manifest these effects together with a high level of aerobic glycolysis which appears to result from loss of control of the balance between glycolysis and (aerobic) respiration, mediated through a NADH-dependent mechanism.

7113 CYCLIC AMP AND MALIGNANT TRANSFORMATION.

(E.) Pastan, I. (Natl. Cancer Inst., Bethesda, Md.) and W. B. Anderson, R. A. Carchman, M. C. Willingham, T. R. Russell and G. S. Johnson. *Cold Spring Harbor Conf Cell Proliferation* 1:563-570, 1974.

Results of a series of experiments on the role of cyclic AMP (cAMP) in malignant transformation are reported. Treatment of transformed cells with dibutyryl cAMP produced an increase in adhesiveness to substratum and a decrease in motility. Normal rat kidney (NKR) cells transformed by the Kirsten strain of murine sarcoma virus looked almost normal after this treatment. The effect of dibutyryl cAMP on adhesiveness or cellular morphology did not require new protein synthesis since these responses were not observed in cells treated with cycloheximide. cAMP levels measured in normal and transformed cells showed a good correlation with cell doubling time. Rapidly growing cells had low cAMP levels and slowly growing cells had high levels. After density-dependent inhibition of growth, cAMP levels were particularly high in 3T3 cells, NKR cells, and human fibroblasts; in contrast, levels failed to rise in transformed cells even when grown to very high densities. All agents that stimulated contact-inhibited cells to grow also lowered cAMP levels, while some conditions which inhibited cell growth raised cAMP levels. Transformation of chick embryo cells with either the Bryan high titer strain (RSV-BH) or the Schmidt-Ruppin strain (RSV-SR) of Rous sarcoma virus led to a fall in cAMP and a decrease in adenylate cyclase activity. With a temperature-sensitive RSV-BH mutant, the fall in cyclic AMP, inactivation of adenylate cylase, and expression of the transformed phenotype was very rapid. Cells transformed by an RSV-SR mutant required some hr to develop changes in all three parameters. It is concluded that some viruses introduce a single transforming gene into the cell, different viruses having different transforming genes. The products of these transforming genes modify cAMP levels in different ways, but each modification leads to decreased cAMP levels. As a consequence, growth rate increases, the cell shape changes, and adhesiveness is decreased.

7114 CYCLIC AMP AND THE DIFFERENTIATION OF NEUROBLASTOMA CELLS IN CULTURE. *(E.)*

Prasad, K. N. (Univ. Colorado Med. Cent., Denver) and S. Kumar. *Cold Spring Harbor Conf Cell Proliferation* 1:581-594, 1974.

The regulation of differentiated functions was investigated in human and mouse neuroblastoma cells in culture. In the mouse, analogs of adenosine 3',5'-cyclic monophosphate (cAMP), prostaglandin (PGE$_1$) and phosphodiesterase (PDE) inhibitors caused morphological differentiation of neuroblastoma cells as shown by the formation of long neurites and by an increase in size of the nucleus and cytoplasm. Cells of most clones were sensitive to cAMP in causing morphological differentiation. Tyrosine hydroxylase (TH) was markedly increased in mouse by some analogs of cAMP and papaverine. The morphologically differentiated neuroblastoma cells induced by X-ray, serum-free medium, and cytosine arabinoside showed no change in TH levels. Sodium butyrate, which inhibits cell division without causing morphological differentiation, increased the enzyme activity. Human neuroblastoma cell cultures showed morphological differentiation after treatment with dibutyryl cAMP (2.0 mM), papaverine (1-10 µg/ml) and PGE$_1$ (10 µg/ml). The time of expression and extent of differentiation were concentration-dependent. The combination of PGE$_1$ (10 µg/ml) and papaverine (2.5 µg/ml) allowed the expression of the differentiated phenotype much earlier and to a much greater extent than that produced by each individual agent. Among all cyclic AMP agents, papaverine was most potent in causing morphological differentiation. Sodium butyrate induced neurite formation in a dose-dependent fashion. This is in contrast to mouse cells in which sodium butyrate reversibly inhibits cell division. Serum-free medium and 5-bromodeoxyuridine caused morphological differentiation similar to that observed in mouse cells. The results indicate that cAMP appears to be involved in the differentiation of mouse neuroblastoma cell cultures. Preliminary data indicate cAMP may play a similar role in human neuroblastoma cell cultures.

7115 METABOLIC CONTROLS IN PRECANCEROUS LIVER. VI. STUDIES *IN VIVO* AND *IN VITRO* ON FEEDBACK REGULATION OF Δ-AMINOLEVULINIC ACID SYNTHETASE IN ETHIONINE-FED RATS.

(E.) Edwards, A. M. (Dep. Biochem. Anim. Physiol., Univ. Adelaide, Australia) and J. R. Sabine. *Eur J Cancer* 10(12):793-800, 1974.

Hepatic δ-aminolevulinic acid (ALA) synthetase activity was studied in normal, cancerous, and ethionine-fed rats following induction by allylisopropylacetamide (AIA) and/or repression by heme (hemin) in order to determine if normal feedback controls are affected in cancerous tissues. Normal, 0.25% D-L-ethionine fed, and Morris hepatoma (5123C, 7794A, and 9618A) implanted Buffalo or Hooded Wistar rats were studied. ALA synthetase induction and repression studies were carried out *in vivo* and *in vitro*. ALA synthetase activity was induced *in vivo* in all animals except those bearing 9618A and 7794A Morris hepatomas. Heme injections *in vivo* decreased AIA induction of activity in all animals except those fed ethionine and those bearing 5123C tumors. Tyrosine aminotransferase activity was also measured in normal and ethionine-fed rats, and although initially higher in ethionine-fed rats, AIA and heme treatments caused similar changes in both groups. In contrast to findings *in vivo* for ethionine-fed rats, heme repression was found to be fully operative when studied *in vitro*, i.e. in liver cells isolated from ethionine-treated rats. The results indicate that cancerous and pre-cancerous liver tissues exhibit a loss of feedback control of ALA synthetase.

7116 PURIFIED MULTIPLICATION-STIMULATING ACTIVITY FROM RAT LIVER CELL CONDITIONED MEDIUM: COMPARISON OF BIOLOGICAL ACTIVITIES WITH ALF SERUM, INSULIN, AND SOMATOMEDIN.

(E.) Smith, . L. (McArdle Lab. Cancer Res., Univ. Wisconsin, adison) and H. M. Temin. *J Cell Physiol* 84(2):181-92, 1974.

o determine what elements are necessary for the initiation of DNA synthesis in cell proliferation, a comparison was made between the purified multiplication-stimulating activity (MSA) factor isolated from line of rat liver cells (CRL) and calf serum in nitiating DNA synthesis in chick embryo fibroblasts. lthough both the CRL MSA and calf serum initiated NA synthesis, a qualitative difference was seen when the substances were removed before actual synthesis had begun. When the CRL MSA was removed, DNA synthesis proceeded only in cells that were in the S phase; the SG1 phase was omitted as opposed to cells cultured with the calf serum. Although purified CRL MSA could support cell multiplication, an increased rate of multiplication of the chick fibroblasts was observed when serum was added; and the addition of serum was necessary for the multiplication of Rous sarcoma virus (RSV)-transformed chick cells. Glucose uptake was directly correlated with DNA synthesis when incubated with CRL MSA, but not with calf serum, suggesting that other factors affecting glucose uptake are present in the serum. Therefore, glucose appears to be a possible primary regulatory factor in DNA synthesis. Other studies showed that survival factors are not related to DNA synthesis, and that CRL MSA was similar in activity to some growth hormone-dependent polypeptides (amorphous porcine insulin and somatomedin) found in human plasma which inhibit non-suppressible insulin-like activity. Although other mitogens have been shown to possess insulin-like activities, it cannot be generalized that this is true of all MSA factors since some, such as epidermal growth factor, do not have insulin-like activity.

7117 INFLUENCE OF ADENOSINE DIPHOSPHORIBOSYLATION OF HISTONES ON THE REPLICATION OF HUMAN CELLS.

(E.) Smulson, M. E. (Georgetown Univ., Sch. Med. Dent., Washington, D.C.). *Natl Cancer Inst Monogr* 38:205-211, 1973.

The activity of polyadenosine diphosphoribose (poly-ADPR) polymerase was studied during various stages of a synchronized human cell cycle to determine whether ADP-ribosylation of nuclear porteins is a normal control mechanism of the eukaryotic cell for controlling mitosis. HeLa S3 cells were synchronized between the G_1 and S phases by the double thymidine block method; the cells were then allowed to proceed through the cell cycle and the synthesis of macromolecules was studied. The specific activity of poly-ADPR polymerase was minimal during the late S phase of one cycle and during early S phase of the second cycle, and was high during mitosis and G_1, periods when scheduled DNA synthesis does not occur. In contrast, the activity of RNA polymerase was low during the M and early S phases, and higher during the late S and G_1 phases. The activity of poly-ADPR polymerase was also examined during the asynchronous cycle of a population of HeLa cells. Maximal ADP-ribosylation of nuclear proteins occurred when the cells had reached the late logarithmic or stationary phase of cell growth, when DNA synthesis was greatly diminished. When DNA synthesis was rapidly re-initiated by cell dilution, the specific activity of poly-ADPR remained at the resting level. The activity of this enzyme also remained low during phytohemagglutinin (PHA) stimulation of DNA synthesis in human lymphocytes. However, the specific activity of the enzyme increased ten-fold by 72 hr and 20-fold by 96 hr in these cells. When intact HeLa cells were incubated with 1 mM dibutyryl cyclic AMP, DNA synthesis was stimulated by 25%, and nuclear RNA polymerase and poly-ADPR polymerase were stimulated by 56% and 30%, resp. The activity of poly-ADPR polymerase was relatively unaffected by the inhibition of protein synthesis in intact HeLa cells, but was stimulated by actinomycin D inhibition of RNA synthesis. The inhibitory effect of actinomycin D on this enzyme occurred only in the intact cell. This data offers no evidence that poly-ADPR polymerase is involved with a chalone mechanism in HeLa cells.

7118 PROTEIN SYNTHESIS WITH RIBOSOMES FROM NORMAL AND MALIGNANT *CERVIX UTERI* AND A NON-ANALOGOUS AMINOCYL-tRNA. *(E.)*

Dube, D. K. (Univ. Coll. Sci., Calcutta, India), S. Chakrabarti and S. C. Roy. *Indian J Biochem Biophys* 11(2):95-96, 1974.

The incorporation of ^{14}C-phenylalanine into protein by a cell-free system derived from normal or malignant human cervical ribosomes is described. The complete system required ^{14}C-phenylalanine-charged tRNA from calf liver, poly U, a "pH 5 enzyme fraction" from the same source as the ribosomes and other cofactors as GTP, ATP, PEP and its kinase. The system was not inhibited by the fungal antibiotic "Jawaharene" or mitomycin C but was inhibited by emetine (2×10^{-4} M). In the complete system, malignant cervix ribosomes incorporated ^{14}C-phenylalanine 10-15% more than normal cervix ribosomes. The increased activity of the system containing ribosomes from malignant cervix is related to the tendency for increased protein synthesis in tumor tissue.

7119 DISTRIBUTION OF TRANSCRIBABLE DNA SEQUENCES IN MOUSE LIVER HEPATOMA CHROMATIN. *(E.)*

Duerksen, J. D. (Univ. Calgary, Canada). *Mol Cell Biochem* 4(3):197-203, 1974.

Sheared chromatin from mouse TLT hepatoma nuclei was used to study the distribution of transcribable DNA sequences. Chromatin was sheared in either a Virtis small or large volume capacity cup and was fractionated on agarose exclusion chromatography columns. *In vitro* incorporation of ^{3}H-uridine triphosphate into acid insoluble material using fractionated chromatin as template was greatest for the large and small-sized chromatin fragments and was relatively low for intermediate-sized fragments which constituted the majority of the total fractions. The differences in template activity among the three groups varied inversely with the shear time. There was no correlation between template activity and protein/DNA or RNA/DNA ratio within a given fraction. It is concluded that the discriminatory ability of shearing to separate the relatively small areas of transcribable chromatin is lost with prolonged shearing times.

7120 DNA REPAIR AND ALKYLATION-INDUCED TOXIC, MUTAGENIC, AND CYTOLOGICAL EFFECTS IN MAMMALIAN CELLS. *(E.)*

Roberts, J. J. (R. Cancer Hosp., Buckinghamshire, England), J. E. Sturrock and K. N. Ward. *Biochem Dis* 4(Part A):401-425, 1974.

Investigations were undertaken to determine whether DNA repair processes restore the original DNA base sequence or only restore the DNA template sufficiently for DNA replication to occur and for the cell to survive, but with its DNA modified so as to lead to subsequent base sequence errors during DNA transcription and hence to mutations. To examine the latter possibility, the effect of caffeine (which interferes with the repair of alkylated DNA in mammalian cells) on the frequency of mutation to 8-azaguanine resistance produced by N-methyl-N-nitrosourea in Chinese hamster cells was investigated. Caffeine did not decrease the mutation frequency, indicating that mutation arise in mammalian cells as a consequence of inadequate DNA repair. This mutation system was further used to determine whether mutations are related to the level of reaction with DNA or to the nature of the products of reaction with DNA. Again, the results indicated that mutation correlated with the cytotoxic actions of the three methylating agents, N-methyl-N-nitrosoure methyl methanesulfonate, and MNNG; therefore, lethal and mutational damage to DNA appear to be manifestations of the same phenomenon. It further follows that cell division must take place before mutation can occur and that cells which are more sensitive to the toxic effect on a particular agent might also be more readily mutated by it.

7121 DNA CONTENT IN CERVICAL CANCER CELLS. *(Rus.)*

Avtandilov, G. G. (Inst. Hum. Morphol., Moscow, USSR), S. S. Kasab'ian and O. V. Sgibneva. *Vopr Onkol* 20(8):14-17, 1974.

Microspectrophotometric studies were performed on 36 cervical tumors from patients aged 36-39 years who had preinvasive cancer (four cases), keratinized squamous cell carcinoma (five), nonkeratinized squamous cell carcinoma (12), poorly differentiated cancer (ten) and adenoma (five). The DNA content (N) was determined in 50 cell nuclei. To determine the amount of DNA corresponding to a diploid set of chromosomes (2N), DNA was measured in the nuclei of 30 lymphocytes in the same sections. Paraoctoploid and polyploid cells accounted for 30% and 5%, resp., of all cells in preinvasive cancer; 20% and 1.6%, resp., in keratinized squamous cell carcinoma; 16.3% and 0.4%, resp., in nonkeratinized carcinoma; 50% and 33.2%, resp., in adenogenous cancer; and 36.8% and 15.6%, resp. in poorly differentiated cancer. In the normal multilayer squamous epithelium of the uterine cervix, paradiploid and paratetraploid cells account for 97% of all cells and paraoctoploid, for only 3%. It is suggested that hyperdiploid cell clones are indicative of cervical tumors. The most characteristic symptom of malignancy is a substantial increase in paraoctoploid and polyploid cells.

7122 MEMBRANE GLYCOPEPTIDES FROM SUBCELLULAR FRACTIONS OF CONTROL AND VIRUS-TRANSFORMED CELLS. *(E.)*

Buck, C. A. (Div. Biol., Kansas State Univ., Manhattan), J. P. Fuhrer, G. Soslau and L. Warren. *J Biol Chem* 249(5):1541-1550, 1974.

The glycopeptides released from various subcellular fractions isolated from control, BHK$_{21}$/C$_{13}$, and virus-

ransformed C_{13}/B_4 baby hamster kidney cells were
ompared on columns of Sephadex G-50 in order to de-
ermine whether the glycopeptides derived from glyco-
roteins associated with subcellular fractions re-
embled those found on the surface membrane of these
ame cells. Cells were grown in the presence of D-
^{14}C]- or [^{3}H]glycosamine or L-[^{14}C]- or [^{3}H]frucose.
he cells were disrupted, and the nuclei were pre-
ared by two methods, one using detergent to dissolve
he outer nuclear membrane and other debris, and the
ther using the effect of shear forces to clear away
ontamination. Fractions to be compared were mixed
nd digested with pronase, and the glycopeptides
ere fractionated by gel filtration. The alterations
ccompanying transformation seen in the surface mem-
rane glycopeptides were also found in other sub-
ellular fractions. These alterations consist of a
arked increase in the amount of higher molecular
eight material in the transformed cell. Neuramini-
ase treatment of this material diminished or elim-
nated the difference between the normal and malig-
ant patterns. The relative distribution of glyco-
eptides in the mitochondrial, nuclear and rough en-
oplasmic reticular fractions differed in some re-
pects from that of the surface membrane. Glyco-
eptides associated with the subcellular fractions
ere not due to contamination by surface membranes.

123 CYTOPHOTOMETRICAL DETERMINATION OF CELLULAR DNA CONTENT IN EXPERIMENTAL BRAIN TUMORS.
. TRANSPLANTED STRAINS OF BRAIN TUMORS FROM LABORA-
DRY ANIMALS.

(Rus.) Khalanskii, A. S. (Inst. Human
orphol., Moscow, USSR). *Tsitologiia* 15(7):912-919,
973.

ytophotometric studies of DNA content were made in
ive transplanted malignant brain tumors in mice (two
pendymomas, one undifferentiated oligodendroglio-
lastoma, glioblastoma multiforme, and angioarach-
oidal endotheliosarcoma); two transplanted tumors
n rats (protiplasmic astrocytoma and glioblastoma
ultiforme); and one in rabbit (undifferentiated as-
rocytoma). The findings indicated that the strains
f the brain tumors varied greatly in the DNA con-
ent. There were predominantly diploid tumors (pro-
oplasmic rat astrocytoma and unidfferentiated rabbit
strocytoma); tetraploid (murine oligodendroglioblas-
oma and glioblastoma multiforme); tumors with
wo modal classes (murine ependymoblastoma); and
umors with no clear stem line (rat glioblastoma
ultiforme and arachnoidal endotheliosarcoma). A
orrelation was observed between the degree of malig-
ancy and ploidy: all transplanted highly malignant
umor strains in mice and rats were hetero- and tetra-
loid. The most malignant tumors had such great var-
ations in the nuclear DNA that their ploidy could not
e determined. The astrocyte series of tumors had
he most constant DNA content.

24 PREFERENTIAL METHYLATION OF REGULATORY GENES IN HELA CELLS.

(E.) Volpe, P.
nt. Inst. Genet. Biophys., Naples, Italy) and T.
emenko. *FEBS Lett* 44(2):121-126, 1974.

he involvement of regulatory genes in methylation
was studied in HeLa S3 cells which were grown in suspension and synchronized with a double thymidine block. DNA extracted during the early S-phase was methylated to a greater degree than that extracted during the late S-phase. The methylation of DNA in the early S-phase was much higher than expected from GC content of the already synthetized DNA. Thus the degree of methylation of DNA seemed to depend not only on the proportion of CG pairs, but C in the euchromatic fraction of DNA synthesized in early S-phase was more methylated than in the heterochromatic AT-rich DNA synthesis in late S. When the methylated DNA was hybridized with the nuclear giant dRNA molecule, an appreciable amount of ^{3}H counts, signifying DNA methylation, were found in the hybrids. When, instead, the same methylated DNA was hybridized with polysomal mRNA, practically no significant ^{3}H counts were found in the hybrids. Thus, the DNA region containing methylated bases was left out by mRNA.

7125 NOVIKOFF HEPATOMA NUCLEAR 8-18S RNA.

(E.) Savage, H. E. (Dep. Pharm., Baylor Coll. Med., Houston, Tex.), V. Grinchishin, W-F. Fang and H. Busch. *Physiol Chem Phys* 6(2):113-126, 1974.

The 8-18S nuclear and nucleolar preparations of RNA from Novikoff ascites tumor cells were isolated on sucrose gradients for nucleotide analysis to ascertain whether these bands are related to ribosomal RNA synthesis or to messenger RNA synthesis. The cells used were incubated with ^{32}P-orthophosphate for periods of six hr for nucleolar RNA preparations and 8-16 hr for nuclear RNA preparations. The 8-18S region was obtained by fractionating the sucrose gradients to include one-third of the downward slope of the 4-8S RNA region and one-third of the upward slope of the 18S RNA. The 8-18S regions contained 9.0% and 9.5% of the total nucleolar and nuclear RNA, respectively. The nuclear 8-18S region was separated into approximately 35 bands by electrophoresis on 4.6% neutral polyacrylamide gels containing 6 M urea. Nucleolar 8-18S RNA had a similar banding pattern. Isolated nuclear or nucleolar RNA bands had similar mobilities on reruns. Using the Millipore filter technique, a filter-bound RNA fraction, comprising only 4% of the total 8-18S nuclear RNA, was found to contain 33.5% AMP, which was much higher than the AMP content of any of the RNA bands found on gel electrophoresis. The nucleotide analyses showed that gel electrophoresis bands were mainly rich in GMP and CMP and low in AMP and UMP. Based on nucleotide composition, there were at least two groups of RNA bands, those with AMP contents of approximately 15%, such as bands 19 and 20, and those with AMP contents of 20%, such as bands 4, 22, and 32. The AMP and UMP contents of the slower migrating RNA bands following band 22 tended to increase. Competitive hybridization experiments indicated that whole nuclear 8-18S RNA contains sequences found in 18S and 28S rRNA, nucleolar 45S RNA, as well as 4-7S nuclear RNA. The presence in the electrophoretically fractionated 8-18S RNA of components rich in GMP and CMP, together with the results of the hybridization experiments, suggest that such components are nonconserved fragments of preribosomal high molecular weight RNA or transcriptional intermediates in the synthesis of preribosomal RNA. It appears, therefore, that meaningful electrophoretic analysis of premessenger or messenger RNA will require prior removal of contaminating preribosomal or ribosomal RNA fragments.

7126 RNA OF LYMPHOCYTES FROM CHRONIC LYMPHOCYTIC LEUKEMIA. *(E.)* Rozynkowa, D. (Acad. Med., Lublin, Poland) and J. Wojcierowski. *Arch Immunol Ther Exp (Warsz)* 22(5):607-618, 1974.

The rates of RNA synthesis in normal and leukemic human lymphocytes were compared in the presence and absence of phytohemagglutinin. Leukemic and control lymphocytes were incubated with a large dose of ^{3}H-uridine and examined by radioautography. The specific activity of newly synthesized RNA of leukemic lymphocytes cultured with ^{32}P-orthophosphate (5 μCi/ml) as a precursor of nucleic acid synthesis was assayed. The specific activities of various fractions of the RNA at fixed times of incorporation of label were compared. These results were compared with data from previous studies on normal lymphocytes. Leukemic cells, on the average, had a lesser incidence of label than normal cells. The specific activity of RNA in various fractions was substantially lower than in the analogous specific activity of RNA of normal lymphocytes. Phytohemagglutinin stimulation of RNA synthesis was far less in leukemic than normal cells. After ^{32}P-labeling of leukemic and normal cells (4.5 hr exposure) and fractionation of extracted RNA on methylated albumin columns, RNA synthesis was depressed compared to normal cells, especially in the ribosomal RNA frations. It is concluded that populations of human leukemic lymphocytes have a slower average intrinsic RNA synthesis rate and lower responsiveness to phytohemagglutinin than normal, but this property may reside only in a small proportion of the leukemic cell population.

7127 AUTORADIOGRAPHIC STUDY, USING AN ELECTRON MICROSCOPE, OF NUCLEOLI IN EHRLICH TUMOR CELLS. DEMONSTRATION OF DNA IN THE CENTER OF FIBRILS. *(Fr.)* Goessens, G. (Inst. Histol., Univ. Liege, Belgium). *C R Acad Sci* [*D*] *(Paris)* 279(12):991-993, 1974.

In vitro cultures of hypertetraploid Ehrlich tumor cells were exposed for varying lengths of time to tritiated actinomycin D, thymidine, uridine, and leucine and were examined by autoradiography. Tritiated actinomycin D and thymidine were found to be present in the fibril centers, confirming the results of previous studies that the fibril centers contain DNA. Leucine was found to be incorporated into the fibril centers after 5 min of contact, confirming the finding that the fibril centers are essentially composed of proteins. Tritiated uridine was incorporated virtually exclusively by the dense fibril component of the nucleoli after five min of contact, corresponding to the localization of RNA synthesis. Coupled with the observations that the dense fibril components are located around the fibril centers, that the fibril centers persist throughout mitosis and that the nucleoli reconstitute themselves about them at the end of telophase, this is taken to show that the fibril centers in nucleoli of Ehrlich tumor cells are the organizers of the nucleoli.

7128 RIBOSOMES, INITIATION FACTORS, AND THE REGULATION OF GENE EXPRESSION. *(E.)* Schapira, G. (Inst. Mol. Pathol., Paris, France), J. Delaunay, D. Bogdanovsky, L. Reibel, C. Vaquero and F. Creusot. *Proc Int Symp Princess Takamatsu Cancer Res Fund, 4th,* 37-54, 1973.

Studies performed shortly after the discovery of mRNA showed that guinea-pig reticulocytes accepted the information contained in the pH 5 enzyme fraction from rabbit reticulocytes and *vice versa*; this was demonstrated by first isolating labeled hemoglobin, then by isolating tyrosine-containing peptides. As a result, the first translation of mRNA from a rabbit allelic for one hemoglobin (αT IV Leu) on ribosomes from another one (α T IV Val) was established. Subsequent experiments demonstrated that fish and plants are able to accept mRNA from rabbit reticulocytes. On the other hand, there is proof of ribosomal specificity. Ribosomes from duck reticulocytes preferentially accepted mRNA from rabbit rather than from duck; ribosomes accept nuclear RNA from the same origin; RNA from free polysomes can stimulate amino acid incorporation in a system with free polysomes; the Mg dependence of ribosomes is different according to the source tissue; and there is a barrier between prokaryotes and eukaryotes as far as the biosynthesis of β-galactosidase in *E. coli* is concerned. In prokaryotes, one protein is necessary to control sensitivity to streptomycin and translational fidelity. Ontogeny and oncogeny, as investigated by two-dimensional gel electrophoresis of ribosomal proteins, showed no differences. Differences between ribosomal protein tissues of the same animal were observed but were minimal. Coelectrophoresis of proteins from rabbit ribosomes mixed with the ribosomal proteins from other species showed no difference between mammals, birds, and reptiles. Going further down the phylogenic tree, changes appeared and increased proportionally as the species became more distant from the rabbit. However, there were still about 25 proteins with the same electrophoretic migration in species as distant as rabbits and plants. These results suggest that a set of ribosomal proteins conserved during evolution may account for the possibility of translation on ribosomes from very distant species. Initiation factors and interference factors are not always totally contained in ribosomes. This is shown by the exchangeability of initial factors between prokaryotes and eukaryotes, and the role of a new RNA (molecular wt 10,000) which links protein initiation factors and mRNA.

7129 RIBOSOMAL RNA SYNTHESIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA. *(E.)* Billington, R. W. (Christie Hosp. and Holt Radium Inst., Manchester, England) and R. F. Itzhaki. *Br J Cancer* 29(4):318-323, 1974.

Mature ribosomal RNA production in chronic lymphocytic leukemia (CLL) lymphocytes was examined using methyltion and (^{3}H)-uridine labeling. Normal controls were atched according to age. Lymphocytes (1-5 x 10^{7}), repared by the Ficoll/Triosil gradient technique were ncubated in 2 ml of TL199 + 10% autologous serum for 0 min before addition of 5 μCi/ml (^{3}H)-uridine (6.3 i/mM). For methylation, cells were incubated in inimal essential medium with Earle's salts, without ethionine, and then 10 μCi/ml of (^{3}H-methyl)-methonine (9 Ci/mM) was added. Total DNA and RNA were stimated and the labeled RNA was extracted. RNA as separated in 2.4% polyacrylamide gels and then ounted in a Beckman Liquid Scintillation counter. ridine incorporation into RNA was consistently highr in leukemic cells. The rate of synthesis of the arious components of RNA was examined. Over short abeling periods, a high molecular weight RNA was ynthesized; this RNA produced more uridine labeling n the CLL than in the normal cell. Normal lymphoytes had a higher proportion of total counts in ature ribosomal RNA as incubation continued. In ymphocytes with a one hr pulse in (^{3}H)-uridine the nitial incorporation was less in normal cells with irtually all precursor processed after six hr. Afer 24 hr, both cell types had similar amounts of adioactivity with some precursor still present in he CLL cells. CLL lymphocytes made large amounts f stable, rapidly labeled high molecular weight RNA hich, in normal cells, was unstable and rapidly rocessed into 28S and 18S ribosomal RNA. Methyltion was normal, indicating little entry of the high olecular weight material into the ribosomal system, hich suggests (a) a normal precursor, but overprouced; (b) an aberrant, unprocessable precursor; (c) o precursor, but possibly HnRNA. Normal lymphocytes ad twice as many ribosomes used in protein syntheis than CLL cells, suggesting a deficit in the proein synthesizing mechanism of CLL cells that is due o faults in polysome assemblage or to a shortage of essenger RNA.

130 MEMBRANE-BOUND POLYRIBOSOMES IN HELA CELLS: ASSOCIATION OF POLYADENYLIC ACID WITH MEMRANES.

(E.) Milcarek, C. (Dep. Biol., Massachusetts nst. Technol., Cambridge) and S. Penman. *Mol Biol* 9(2):327-338, 1974.

he poly(A) sequences from the mRNA isolated from oth the loose and tight membrane-associated ribosomes f HeLa cells were investigated. HeLa cells grown in uspension culture were fractionated and the membrane-ound material sedimented on sucrose gradients. The olyadenylic acid of the membrane-bound polyribosomes as associated with rapidly sedimenting membrane tructures. Most of this poly(A) remained associated ith the membranes after extensive degradation of the olyribosomal mRNA with pancreatic ribonuclease; 57% f the membrane-associated poly(A) still sedimented ith the membrane structures after ribonuclease treatent followed by the addition of EDTA. Reconstrucion experiments suggest that the poly(A) from deterent-treated, membrane-derived polyribosomes was not rapped by other membrane structures. The poly(A)-ontaining RNA fragment, which remained associated ith the membranes after pancreatic ribonuclease reatment, sedimented as a single peak at about 7S, eing about twice the size of cellular poly(A). Thus, the attachment site was almost pure poly(A). The poly(A)-containing, membrane-bound mRNA appeared to be of a larger average size than the total cellular poly(A)-containing RNA, as indicated by its greater sedimentation value.

7131 RNA METABOLISM IN NEUROBLASTOMA CULTURES. 1. RIBOSOMAL RNA.

(E.) Casola, L. (Int. Inst. Genet. Biophys., Naples, Italy), M. Romano, G. Di Matteo, G. Augusti-Tocco and M. Estenoz. *Dev Biol* 41(2):371-379, 1974.

The synthesis, processing, and degradation of ribosomal RNA was investigated in neuroblastoma cells in suspension and monolayer cultures. The incorporation of ^{14}C-uridine into RNA and the specific activity of uridine triphosphate (UTP) were used to measure the rate of RNA synthesis. When monolayer and suspension cells were incubated with ^{14}C-uridine (5 μCi/ml) for varying periods of time and incorporation of uridine was measured on perchloric acid-insoluble RNA fractions, it was found that the rates of synthesis of RNA were the same for both types of cells for the first hr. After one hr the rate of uridine incorporation in suspension cells leveled off while that in monolayer cells increased. This difference may have been due to differences in RNA contents of the 2 types of cells, since previous work showed that monolayer neuroblastoma cells may contain twice the RNA content of corresponding cells grown in suspension. Labeling with ^{3}H-methionine under the above conditions indicated similar rates of 45S preribosomal RNA (pre-rRNA) in the two cell types. However, the ratio of radioactivity in 28S RNA relative to 45S RNA (which was calculated as cpm in 28S/cpm in 45S) was found to be much higher in monolayer than in suspension cells. The following ratios were recorded for monolayer and suspension cells resp.: one hr incubation (0.78 and 0.31), two hr incubation (1.25 and 0.67), and three hr incubation (1.57 and 0.46). This indicated a higher rate of processing of pre-rRNA and transport of rRNA into the cytoplasm in the monolayer cells. The decay of monolayer and suspension cells (4 x 10^{6} cells) labeled with 15 μCi/ml ^{3}H-methionone in the presence of adenosine, guanosine, and thymidine (2 x 10^{-5} M) for 18 hr and chased with cold methionine (2 x 10^{-2} M) occurred at nearly equal rates. The higher rRNA content in neuroblastoma cells growing in monolayer in contrast to cells in suspension is due neither to an increased rate of synthesis of rRNA precursor molecules nor to a slower turnover rate of the product. The results indicate independent synthesis and cleavage mechanisms of the pre-rRNA molecules.

7132 SOME PROPERTIES OF "LIGHT" CYTOPLASMIC RIBONUCLEOPROTEIN OF MOUSE PLASMACYTOMA.

(E.) Grechko, V. V. (Inst. Mol. Biol., Acad. Sci., Moscow, USSR), N. K. Saccharova and L. E. Gorjunova. *Eur J Biochem* 47(1):67-74, 1974.

The sedimentation density and kinetics of the cytoplasmic RNA protein particles from mouse plasmacytoma MOPS$_{406}$ were studied after cell wall disruption. After 1-3 hours of incubation with [5-^{3}H] uridine, greater than 50% of the radioactivity was found in the postribosomal region of the sucrose gradient; there was practically no radioactivity of nascent peptides in the monoribosomal peak or the postribosomal gradient region. The cytoplasmic RNA protein particles were stable to the action of sodium deoxycholate; this detergent was able to act in the presence of Triton X-100. Density measurements on CsCl gradients showed the presence of two types of particles in the cytoplasmic postribosomal radioactivity: one with a density similar to that of nuclear particles and the other with a density suggesting that they contained larger amounts of RNA. The sucrose gradient pattern of the labeled RNAs present in the light RNA protein particles was similar to that of RNA from polyribosomes. The incorporation of label into the nuclear RNAs increased linearly with time; for the ribosomal RNAs and postmitochondrial supernatant RNAs, the incorporation curves showed a lag period, indicating that the appearance of label in these RNAs was secondary to its appearance in the nuclear RNAs. Thus, nonribosomal RNA seemed to appear naturally in the cytoplasm. No mitochondrial particles cosedimented with the ribosomal material in sucrose gradients. The data support the cytoplasmic origin of cytoplasmic postribosomal ribonucleoprotein particles.

7133 THE RELATION OF RIBONUCLEASE-RESISTANT RNA TO DNA SYNTHESIS AND CELL PROLIFERATION.

(Rus.) Kucheriavenko, A. A. (Inst. Human Morphol., Moscow, USSR) and L. V. Van'ko. *Dokl Akad Nauk SSSR* 219(5):1267-1270, 1974.

Spleen cells from male CBA mice immunized with 0.2 ml of 25% sheep RBC suspension were incubated for 1 hr with ^{3}H-thymidine or ^{3}H-uridine. Continuous HeLa cell culture served as a model. Total RNA synthesis occurred in most of the antibody producing, rosette-forming and other lymphoid cells. Treatment with ribonuclease reduced the number of labeled cells. On the third day of the immune response, the plaque-forming cells consisted of 50% blast cells, 30% rosette-forming cells and 4.5% other. Blast cells contained the largest number of DNA-synthesizing cells. In HeLa cells ribonuclease-resistant RNA synthesis began during the G_1 period, reached its peak at the end of the G_1 and beginning of the S periods and stopped at the end of the S period. Thus, RNA synthesis preceded that of DNA.

7134 LIPID ANALYSIS OF CELL NUCLEI IN YOUNG AND OLD EHRLICH ASCITES TUMORS.

(E.) Balint, Z. (Oncopathological Res. Inst., Budapest, Hungary) and L. Holczinger. *Int J Cancer* 14(1):93-96, 1974.

Changes in the lipid composition of ascites tumor cell nuclei is described in this study designed to investigate reports of fatty degeneration of tumor nuclei and lipid accumulation in tumor ascites plasma. Lipid content of nuclei was measured in Ehrlich ascites cells after seven and 14 days of i.p. growth. Contents of RNA, DNA, and protein changed less than 7.5% in either whole cells or nuclei; total lipid increased from 13.8% (dry weight) to 19.5% in whole cells and from 7.3% to 15.0% in nuclei. The largest increases were in the neutral lipids (cholestrol ester, cholesterol, free fatty acid, and triglycerides); triglycerides increased 9-fold in the nucleus while phospholipids remained constant. While the significance of these changes is not clear, they do coincide with an increase in cell cycling time, a decrease in the proliferating fraction of the cell population, an increase in cell loss and other aspects of the plateau phase of ascitic tumor growth.

7135 ENZYME PATTERNS IN NON-NEOPLASTIC AND SPONTANEOUSLY TRANSFORMED TISSUE CULTURE CELLS: A HISTOCHEMICAL AND BIOCHEMICAL STUDY.

(E.) Wilson, P. D. (Imp. Cancer Res. Fund., London, England). *J Pathol* 114(1):21-28, 1974.

Enzyme patterns were compared by quantitative biochemical assay and light and electron microscopic histochemistry of seven spontaneously transformed neoplastic and five non-neoplastic long-term tissue culture cell lines with similar morphological characteristics (although derived from various organs of young, old and embryonic C57 and C3H mice). The activity of surface enzymes was strikingly changed in neoplastic lines. Alkaline phosphatase activity was absent or at very low levels; glucose-6-phosphate dehydrogenase, lactate dehydrogenase and 5'-nucleotidase levels were low and β-glucuronidase levels were high in neoplastic lines compared with non-neoplastic lines. Acid phosphatase activity was present in lysosomes but was also demonstrated on the plasma membranes of four tumor-producing and four non-tumor-producing cell lines. The decrease in surface enzymes in the neoplastic cell lines suggested that on acquisition of tumor-producing capacity there was a change in the surface active transport mechanism of the cells. Although the activities of only three enzymes were relatively similar in the neoplastic and non-neoplastic cell lines they were all mitochondrial enzymes. It is suggested that these enzymes undergo their adaptation to the culture system during early passages and then remain constant at later passages. The observation that some cells showed intense succinic dehydrogenase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase activity while adjacent identical cells showed none emphasizes the limitations of biochemical assay. It is concluded that the cells within a culture apparently vary considerably in their state of metabolic activity and biochemical assays of activity must be considered as being average value.

7136 DISTRIBUTION PATTERN OF SEVERAL ENZYMES OF PLATELETS UNDER NORMAL AND LEUKEMIA CONDITIONS.

(E.) Ray, B. (Dep. Biochem., Univ. Coll. Sci., Calcutta, India), B. Basu, D. K. Bhattacharya and G. C. Chatterjee. *Cancer* 34(5):1756-1760, 1974.

The intracellular distributions of enzymes such as acid phosphatase, alkaline phosphatase, β-glucuronidase and succinate dehydrogenase in isolated normal and leukemic platelets were investigated to determin whether the enzyme activities of platelets are also altered under pathologic abnormalities of the leukocytes in leukemic conditions. Platelets from normal and leukemic peripheral blood were isolated by differential centrifugation and homogenized for use in the enzyme assay. Platelet alkaline phosphatase activity in any fraction studied was not changed under leukemic conditions. Increase of acid phosphatase and β-glucuronidase and decrease of succinate dehydrogenase activities were observed in the leukemic platelet homogenate and in other fractions. The specific activities of acid phosphatase and β-glucuronidase increased in the mitochondrial and lysosomal fractions, whereas the specific activity of acid phosphatase decreased markedly. Such activity of β-glucuronidase did not change in the post lysosomal supernatant fractions of leukemic platelets. Specific activity of alkaline phosphatase in any of the above fractions of leukemic platelets did not change. In the case of succinate dehydrogenase, the specific activity of the enzyme in the mitochondrial fraction decreased markedly, but the activity did not change in other fractions of leukemic platelets. It is possible that the controlling system of β-glucuronidase activity in the leukocytes is different from that of the platelets. From the distribution pattern as observed in respect to acid phosphatase and β-glucuronidase activities in different subcellular fractions, it is assumed that leukemic platelet lysosomes are less fragile as compared to normal platelet lysosomes.

7137 KINETIC REGULATION OF ADENYLATE KINASES FROM MUSCLE, LIVER, AND HEPATOMA. *(E.)*

Pradhan, T. K. (Univ. Florida Coll. Med., Gainesville), W. E. Criss and H. P. Morris. *Cancer Res* 34(11):3058-3061, 1974.

The kinetic and physical protein parameters of the major adenylate kinases from rat liver, skeletal muscle, and Morris hepatoma 3924A were compared using homogeneous enzyme preparations. At concentrations of 50 mM, the tumor enzyme was activated by citrate, isocitrate, malate, fumarate, succinate, oxaloacetate, α-ketoglutarate, and cis-aconitate; the liver enzyme was activated by all concentrations of succinate and oxaloacetate, and the muscle adenylate kinase was activated by all but malate, fumarate, and succinate. The activation constants of citrate for the liver, muscle, and hepatoma adenylate kinases were 0.09, 0.26, and 15.8 mM, resp. The apparent Michaelis constants for the liver, muscle, and tumor enzymes were 1.9, 6.2, and 18.0 (for AMP), and 7.0, 10.0, and 33.0 mM (for ATP), resp. Citrate decreased the K_m (ATP) with the liver and muscle adenylate kinases and decreased the K_m (AMP) only with the muscle enzyme. Most of the Hill plot-slope values (for ATP) were near 1.5, indicating partial ligand-ligand cooperation. Only in the presence of citrate with ATP as the variable substrate was the slope value greater than 2.0 for liver adenylate kinase. Of various modulators tested, only the nucleoside 5'-diphosphates stimulated the three enzymes, and the stimulation was only in the direction of ADP production. Free fatty acid irreversibly inhibited each of the purified adenylate kinases. Saturated fatty acids did not alter the activities of the enzymes, while the unsaturated fatty acid, oleic acid, inhibited the enzymatic activity in all cases. Sodium lauryl sulfate inhibited all three enzymes. The denaturation of the enzyme proteins by detergent was not reversible. Mercurial reagents inhibited the activity of each enzyme, but each enzyme appeared to have a slightly different degree of sulfhydryl lability. With p-hydroxymercuriphenylsulfonate, the K_i for liver adenylate kinase alone increased significantly in the presence of citrate; with p-chloromercuribenzoate, the K_i values of the liver and muscle enzymes, but not the tumor enzyme, increased 20-fold in the presence of citrate. In the absence of citrate, the K_i values of the three enzymes were similar with both mercurial reagents.

7138 ISOZYMES OF HEXOSAMINIDASE IN VARIOUS HEPATOMAS. *(E.)*

Weber, A. (Natl. Inst. Health Med. Res., Paris, France) and F. Schapira. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973*, 1:309-314, 1974.

Normal, hepatectomized and cancerous Wistar rats were used to study the isozymic fractionation of hexosaminidase activity; host livers of rats with Reuber hepatomas were also used. The animals were sacrificed six hr to seven days after surgery. Regeneration was also induced by intraperitoneal injection of CCl_4 (100 µl/100 g). Primary, slow-growing and well-differentiated hepatomas were obtained by feeding rats with 600 mg of 3'methyl-4-dimethylaminoazobenzene (3'MeDAB)/kg of diet. Solid-transplanted, fast growing and poorly differentiated hepatomas were induced by feeding 4 dimethyl-aminoazobenzene (DAB) or 0.025% *N*-2 fluorenyldiacetamide. Human hepatomas were obtained by surgical resection. The fluorimetric measurement of 4-methylumbelliferone liberated from 4 methylumbelliferyl 2 acetimido-2-deoxy-β-D-glucopyranoside by cellulose acetate strip electrophoresis allowed quantification of the enzymic activity (expressed as nM of substrate hydrolyzed/g of fresh tissue/min at 24 C. Two principal bands were obtained. In normal rat liver, regenerating liver or CCl_4-intoxicated liver, muscle, lung, brain, red cells, placenta, slow growing rat hepatoma or four different human hepatomas, a weaker anodic band and a stronger cathodic band pattern were observed. In fast-growing rat hepatoma or rat fetal liver tissue the band pattern was reversed. These results suggest that the isozyme pattern relates hepatoma malignancy more to the fetal growth state than cellular proliferation rate.

7139 ENZYMATIC STUDIES OF NORMAL AND MALIGNANT INTESTINAL EPITHELIUM. *(E.)*

Salser, J. S. (Sloan-Kettering Inst. Cancer Res., New York, N.Y.) and E. Balis. *Cancer* 34(3/Suppl.):889-895, 1974.

At attempt was made to determine whether the presumed mitotic regulatory enzyme, thymidine kinase (TK), differs in its action in dividing cells of the normal adult, fetal, and malignant colon. TK is apparently present in a soluble form and a particulate-bound form, both of which are activated by phospholipase C (PC). The soluble enzyme is thought to be responsible for *in vivo* conversion of thymidine to thymidylic acid. In a surgical specimen of normal colon, TK activity of dividing crypt cells was found to be much greater than that of nondividing surface cells; however, in the presence of PC, the surface cells were stimulated more than were the crypt cells so that the total amount of enzyme as revealed by the PC assay was the same in both cases. Compared to the normal specimen, several carcinomas of the gastrointestinal tract generally had more TK activity while fetal intestines did not. TK activity in the presence of PC was not increased in tumors of the stomach, ileum and cecum, but in normal adult tissue was stimulated on the average 2.5-fold and in normal fetal tissue, 7-10-fold. TK heat lability was greater in the tumor preparation than in the normal sample and was more pronounced in the presence of PC. In all cases the slope of the second portion of the biphasic heat inactivation curve was the same. Addition of mercaptoethanol (M) and dithioerythritol (D) to the supernatants increased TK activity more in tumor material than in control; reduced glutathione (G) was inhibitory for both. Gel filtrates of the normal colon showed no TK stimulation with M, D or G, but gel filtrates of the tumor had increased TK activity with all three. Results of studies of heat stability and mercaptan effectors indicate that TK in tumors is not identical to TK in the normal colon. It is suggested that regulation of cell division in gastrointestinal cancer is not identical to that of rapidly dividing crypt or fetal intestinal cells.

7140 AN ULTRASTRUCTURAL STUDY OF ACID PHOSPHATASE ACTIVITY IN NORMAL, ADENOMATOUS AND HYPERPLASTIC (CHIEF CELL TYPE) HUMAN PARATHYROID GLANDS.

(E.) Shannon, W. A., Jr. (Harvard Med. Sch., Boston, Mass.) and S. I. Roth. *Am J Pathol* 77(3):493-506, 1974.

Acid phosphatase activity in a variety of normal, adenomatous and hyperplastic human parathyroid glands was investigated to clarify the secretory process in these glands. In the chief cells of the normal and pathologic human parathyroid glands, nonspecific acid phosphatase activity, demonstrated by hydrolysis of β-glycerophosphate at pH 5.0 was associated with electron-dense membrane-limited inclusions of various sizes, shapes and dimensions, which represent lysosomes which are derived from the Golgi region. There was a different group of smaller, more numerous, more regularly spherical membrane-limited granules which did not contain acid phosphatase also present in the normal parathyroids. It would appear that in the parathyroid glands the smaller granules correspond to the granules containing parathyroid hormone and represent the secretory granules. The electron-opaque granules in the capillary endothelial cells contained acid phosphatase and were more likely lysosomes unrelated to the parathyroid hormones secretory granules. The presence of secondary lysosomes containing ingested secretory type granules in the adenomatous and hyperplastic parathyroid glands suggests that these chief cells are attempting to destroy some of the excess synthesized hormone. It is thus suggested that the variation of the degree of hypercalcemia and serum parathyroid hormone levels in patients with primary hyperparathyroidism is the result of the interplay of four factors: the number of active chief cells, the rate of hormone synthesis by these cells, the rate of hormone secretion by these cells, and the rate of destruction of the synthesized hormone by the lysosomal mechanism.

7141 TERMINAL DEOXYRIBONUCLEOTIDYL TRANSFERASE IN HUMAN LEUKEMIA.

(E.) Coleman, M. S. (Univ. Kentucky Med. Cent., Lexington), J. J. Hutton, P. De Simone and F. J. Bollum. *Proc Natl Acad Sci USA* 71(11):4404-4408, 1974.

The case of a 72-year-old woman (MR) with acute leukemia in whom deoxyribonucleotidyl transferase activity was found in the leukocytes and bone marrow in levels generally seen in the thymus is reported. Samples of bone marrow and peripheral blood leukocytes from this patient, adult patients with acute myelogenous or myelomonocytic leukemia, and non-leukemic patients were assayed for terminal deoxyribonucleotidyl transferase. The enzyme was found at a level of 16 U/10^8 bone marrow cells and 14 U/10^8 circulating leukocytes (1 U = 1 nm nucleotide/hr) in the samples from MR. Assays of transferase in the marrow and peripheral leukocytes of the patients with typical acute and chronic myelogeneous leukemias gave average values of 0.5 and 0.3 U/10^8 cells, resp. Transferase activity was also found in the normal bone marrow samples at levels of about 0.07 U/10^8 cells, while normal peripheral lymphocytes contained less than 0.01 U of transferase activity/10^8 cells. The terminal deoxyribonucleotidyl transferase in all samples of human marrow and peripheral blood showed reaction characteristics, sedimentation characteristics, and chromatographic properties similar to those of the homogeneous enzyme from calf thymus. The results indicate that the occurrence of transferase outside of the thymus is unusual and that a readily detectable level in nonthymic tissue is probably indicative of pathology.

7142 NUCLEIC RIBONUCLEOTIDE REDUCTASE IN THE CELLS OF NORMAL AND REGENERATING LIVER AND SARCOMA M-1 IN RATS.

(Rus.) Sheremetievskaia, T. N. (I. M. Sechenou 1st Moscow Med. Inst., USSR), S. A. Silaieva, B. Ia. Khatsernova and S. S. Debov. *Vopr Med Khim* 20(4):430-435, 1974.

Tests were run on albino male rats to study ribonucleotide reductase activity in the purified cell nuclei of normal liver; regenerating liver 15, 24 and 48 hr after partial hepatectomy; and continuous sarcoma M-1. Ribonucleotide reductase activity in the preparation of normal liver was 1/10 that in tumor cell nuclei. Reduction of cytidine diphosphate into deoxycytidine diphosphate reached a maximum 24 hr after hepatectomy at a time when mitosis began in the proliferating tissue; 48 hr later the specific activity of the enzyme was twice that of normal liver nuclei. The highest specific activity of ribonucleotide reductase was observed in the deoxyribonucleoprotein fraction where it was two to six times the enzymatic activity in intact hepatic nuclei and nine times that in sarcoma M-1 cells. In the tumor cells, ribonucleotide reductase activity in the fractions of whole nuclei and deoxyribonucleoprotein was 0.6 and 2.6 units/mg of DNA, resp. The data obtained suggest the presence of natural inhibitors of regeneration in normal liver. In addition, the ribonucleotide reductase activity is indicative of the physiological state of the tissue under examination.

7143 CELL SURFACE GALACTOSYLTRANSFERASE AND LECTIN AGGLUTINATION OF THYMUS AND SPLEEN LYMPHOCYTES.

(E.) LaMont, J. T. (Harvard Med. Sch., Boston, Mass.), J. L. Perrotto, M. M. Weiser and K. Isselbacher. *Proc Natl Acad Sci USA* 71(9):3726-3730, 1974.

Lectin agglutination and cell surface galactosyltransferase activity were studied with thymus and spleen lymphocytes of neonatal Sprague-Dawley rats. Thymus lymphocytes were more agglutinable by concanavalin A (Con A) than by wheat germ agglutinin, whereas spleen lymphocytes were more agglutinable by wheat germ agglutinin than by Con A. Thymus lymphocytes, but not spleen lymphocytes, of neonatal rats could be blast transformed by Con A (5 µg/ml). Thymus and spleen lymphocytes possessed surface galactosyltransferase activity with both endogenous and exogenous acceptor. The cell surface enzyme for both types of lymphocytes had a pH optimum of 7.5, required Mn^{++} for optimum activity, and was specific for fetuin from which sialic acid and galactose has been removed. Activity with exogenous fetuin acceptor was 3- to 4-fold greater than that with endogenous cell surface acceptor for both spleen and thymus cells. In blast-transformed thymus cells, cell surface galactosyltransferase activity increased markedly from very low pretreatment levels to 1970 cpm/10^7 cells at 72 hr after Con A treatment; in thymus cell cultures that were not blast transformed, there was a negligible increase of activity from 2-72 hr. Spleen lymphocytes showed only minimal changes in galactosyl transferase activity over 72 hr, either with or without Con A. The differences in lectin agglutination suggest a difference in the surface membranes of thymus and spleen lymphocytes. The increase in cell surface galactosyltransferase activity with blast transformation of thymus lymphocytes may be related to the exteriorization of the Golgi apparatus into the plasma membrane.

7144 TEMPLATE CAPACITY OF MOUSE TLT HEPATOMA CHROMATIN FRACTIONS.

(E.) Duerksen, J. D. (Dep. Biol., Univ. Calgary, Canada) and R. J. Smith. *Int J Biochem* 5(11/12):827-844, 1974.

An assay for the template RNA polymerase activity of mouse hepatoma chromatin that had been sheared and fractionated on high molecular weight-excluding agarose columns was described. Some endogenous RNA polymerase activity was found in TLT chromatin but no protease activity was found. Template activity in the presence of *Escherichia coli* RNA polymerase varied greatly with the storage age and purity of the RNA polymerase sample, which was also completely eliminated by the addition of DNase. Conditions causing chromatin reaggregation prior to fractionation (such as a too high buffer concentration of a pH outside 6.5-7.0 range) inhibit activity. The results show the reliability of *E. coli* RNA polymerase in measuring the template activity of DNA and help to resolve differences in the observed template activity of different segments of DNA.

7145 ENHANCING EFFECT OF CHORIONIC GONADOTROPHIN, ESTROGEN HORMONE AND VITAMIN B12 ON TUMOR GROWTH: IMPLICATIONS FOR SPONTANEOUS CANCERS.

(E.) Serrou, B. (Dep. Immunol., St. Eloi Hosp., Montpellier, France), J. B. Dubois, T. Reme, G. Negre, M. Souche, R. Senelar and Cl. Remier. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:315-322, 1974.

The effects of exogenous estrogenic hormones and chorionic gonadotrophic hormones, which are secreted in large amounts during pregnancy, on tumor-like and histological and immunological changes in lymphoid tissues were described. Two rhabdomyosarcomas in the pig and six tumors in dogs (one thyroid epithelioma, one osteosarcoma, one myxosarcoma, one lymphosarcoma, one adenocarcinoma of the breast and one tonsillar epithelioma) were studied. The thymus, metastasis-free lymph nodes and spleen were studied in the animals; in man, the spleen and metastasis-free lymph nodes were examined, after surgical removal, by optical and electron microscopy and by immunofluorescence. Parallels were drawn between facilitation of tumor growth by these hormones and acceptance of a fetus under similar conditions. The mean survival and tumor appearance times of BDF1 mice implanted with Lewis lung tumors were reduced by injection of estrogenic hormone, gonadotrophic hormone or vitamin B12, especially when given 14 days prior to tumor implantation. Histological changes during tumor development, more marked with hormones or vitamin B12, included cortical thymic cysts containing PAS-positive material, followed by thymic atrophy, invasion of red cell regions of spleen by lymphoid cells, splenomegaly and development of giant plasmocyte-type cells laden with immunoglobulins. These histological effects were confirmed in lymph nodes and spleens of spontaneous tumor-bearing pigs, dogs, and humans. A theory for immunological relations between tumor and host, mediated by the thymus, and paralleling those between a developing fetus and its maternal host, was presented.

7146 QUERCETIN, AN ARTIFICIAL REGULATOR OF THE HIGH AEROBIC GLYCOLYSIS OF TUMOR CELLS.

(E.) Soulinna, E. M. (Sect. Biochem., Mol. Cell Biol., Cornell Univ., Ithaca, N.Y.), D. R. Lang and E. Racker. *J Natl Cancer Inst* 53(5):1515-1519, 1974.

The mechanism behind the high aerobic glycolysis observed in tumor cells was studied using a variety of tumor cell lines. Ehrlich ascites cells were maintained in mice, harvested, washed and analyzed; other cell lines were grown *in vitro*. Samples of ATPase were incubated for ten min at 22 C in a solution containing 25 M of *N*-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid buffer (pH 6.7), 10 g pyruvate kinase, and quercetin of F_1 inhibitor. The reaction was initiated, allowed to run for ten min and terminated by the addition of 0.1 ml of 50% trichloroacetic acid. It was then centrifuged and P_i was determined in the supernatant. The results indicated that the high aerobic glycolysis observed in several tumor cells was caused by high ATPase activity. For example, quercetin and fisetin, which inhibit mitochondrial ATPase at low concentrations, virtually stopped lactic acid production in Py-3T3 cells. Morin, a less effective inhibitor of ATPase, was also much less inhibitory to glycolysis, and rutin had little effect on either mitochondrial ATPase or on glycolysis. In some tumors (e.g., Ehrlich ascites cells), the ATPase activity of the Na+K+ pump was responsible for the high aerobic glycolysis; in some (e.g., rapidly growing Py-3T3 fibroblasts), the mitochondrial ATPase was responsible; and in others (e.g., neuroblastoma cells), an unidentified ATPase-generated ADP and P_i were required for glycolysis. In the cells analyzed thus far, the increased ATPase activity was not due to an increased function of the pump, but to a loss of the control mechanism responsible for tight coupling. Some bioflavonoids (e.g., quercetin) inhibited the aerobic glycolysis by specifically repairing the defective control mechanism.

7147 EARLY STAGES IN ESTROGEN CONTROL OF CELL PROLIFERATION. *(E.)*

Bresciani, F. (Fac. Med. Surg., Univ. Naples, Italy), G. A. Puca, E. Nola and V. Sica. *Cold Spring Harbor Conf Cell Proliferation* 1:67-83, 1974.

The early stages of 17β-estradiol interaction with the uterus [(1) association of the incoming hormone with a specific receptor in the cytoplasm, (2) transformation and translocation of the estrogen-receptor complex to the nucleus, and (3) interaction of the complex with chromatin] are reviewed. Studies on calf uterus resulted in the identification of a receptor-transforming factor (RTF) of cytoplasm that acts on the native estrogen-receptor complex (5.3S) to produce a smaller complex (4.5S) which is found in the nucleus. The RTF has been partially purified. It is a protein which sediments at 6.4S, has a Stokes radius of 46 Å, a molecular weight of 115,000, and an isoelectric point of 4.9. It has a high affinity for the native estrogen-receptor complex and shows a Ca^{++}-dependent proteolytic activity on α-casein but not on native or denatured bovine plasma albumin, ovalbumin, or hemoglobin. The pH optimum is 8.7. The RTF-induced size decrease of the native estrogen-receptor complex appears to be a case of limited hydrolysis and may be instrumental in the cytoplasm to nucleus translocation of the protein-bound hormones. To identify nuclear acceptor sites for the estradiol-receptor complex, nuclear components in calf uterus fractions were coupled with CNBr-activated Sepharose. Nuclear binding sites were identified which have an affinity in excess of 10^9 liters/mole (+4 C) for estrogen-receptor complex, but not for free receptor, at 0.1 M KCl. These sites are protein in nature, are soluble at 2 M NaCl, pH 7.1, but precipitate when KCl concentration is decreased to 0.1 M. They are not retained by hydroxyapatite at pH 7.0 but are retained by carboxymethyl (CM)-cellulose at pH 4.0 or 6.0. Hydrochloric acid (0.03 N) is required for their elution from CM-cellulose. These results indicate that the estrogen-receptor-complex acceptor sites consist of basic protein. Protamine, histone IIa (calf thymus), and α-casein coupled to Sepharose also associated with estrogen-receptor complexes but with affinities on the order of 10^2 liters/mole at +4 C. Based on these findings, it is hypothesized that estrogen enters the cell and interacts with the 5.3S receptor, which is confined to the cytoplasm through association with some cytoplasmic structure. Activation of RTF by Ca^{++} results in the production of a mobile 4.5S-estrogen fragment, which enters the nucleus and specifically interacts with a basic acceptor protein.

7148 17-KETOSTEROID EXCRETION OF PATIENTS SUFFERING FROM MAMMARY CARCINOMAS. *(E.)*

Heise, E. (Cent. Inst. Cancer Res. Acad. Sci., Berlin-Buch, East Germany) and M. Gorlich. *Proc Fifth Int Symp Biol Characterization Hum Tumors, Bologna, April 4-6, 1973* 1:208-212, 1974.

The relationship between urinary profiles of excreted 17-ketosteroids and 17-hydroxycortiocosteroids in postmenopausal women (40 mammary cancer patients and 18 controls, mean age about 60 yr) was analyzed. After incubation with β-glucuronidase, the 17-ketosteroids were separated by Girard-reagent; thin layer chromatography was used for further separation. No differences of overall steroid excretion profiles were seen but fractionation into the three 11-deoxy-17-ketosteroids; etiocholanone, androsterone and dehydroepiandrosterone, revealed depression of these steroids in cancer patients. Values were 2589 µg 17-ketosteroids/24 hr for controls and 1788 µg 17-ketosteroids/24 hr for cancer patients, the largest decreases being in dehydroepiandrosterone associated with stage three and four breast cancer. Patients were followed for two yr after mastectomy and a correlation was found between high etiocholanone/dehydroepiandrosterone ratios at mastectomy and subsequent relapse. It is suggested that the hormone excretion level is of prognostic value.

7149 ECTOPIC PRODUCTION OF HUMAN CHORIONIC GONADOTROPHIN BY HUMAN BREAST TUMORS. *(E.)*

Sheth, N. A. (Cancer Res. Inst., Bombay, India), J. N. Saruiya, K. J. Ranadive and A. R. Sheth. *Br J Cancer* 30(6):566-570, 1974.

A large number of cancer patients were screened for human chorionic gonadotrophin (HCG) using a radioimmunoassay method which estimates the amount of the β subunit of HCG is serum. Samples were obtained from 65 breast cacinoma patients, ten cystic mastitis patients, five gynecomastia patients, and seven fibroadenoma patients. Samples for comparative analysis were drawn from normal men, normal pregnant and non-pregnant women, and from choriocarcinoma patients.

Nine of 65 breast carcinoma patients tested HCG positive (10-15 mIU of β-HCG/ml). Cystic mastitis, gynecomastia and fibroadenoma pateints were negative, as were normal men and non-pregnant women. Choriocarcinoma patients and pregnant women tested positive (24.3 ± 4.5 IU and 8.6 ± 1.8, 2.4 ± 0.5, and 4.8 ± 1.2 IU β HCG/ml, resp. The results show the incidence of HCG-secreting breast tumors to be 13%, and indicate the potential use of radioimmunoassay in studying the course of the disease during treatment.

7150 A TUMOUR CELL AGGREGATION PROMOTING SUBSTANCE FROM RAT ASCITES HEPATOMA CELLS.

(E.) Kudo, K. (Kumamato Univ. Med. Sch., Japan), I. Tasaki, Y. Hanaoka and H. Hayashi. *Br J Cancer* 30(6): 549-559, 1974.

A substance capable of promoting tumor cell aggregation was released from rat ascites hepatoma cells (possibly from the cell surface) kept in Hank's balanced salt solution (free of calcium and magnesium) in the cold, and then partially purified by chromatography with DEAE-Sephadex and gel filtration with Bio-gel. The thermostable substance seemed to be a glycoprotein, and its molecular wt was about 72,000 when measured by gel filtration on Sephadex G-200. It has no proteolytic activity. The material was clearly effective in aggregating rat ascites hepatoma cells and simian virus 40 (SV40)-transformed cells, but was less effective for Chang's cells and apparently ineffective for normal rat liver cells and RBC. The action of this material was more potent than that of Jack bean concanavalin A when assayed for aggregation of SV40-transformed cells, the potency of 11 μg of the aggregation promoting factor corresponding to that of 250 μg of Jack bean concanavalin A. The effect of the substance was not influenced by concanavalin A inhibitors such as α-methyl-D-glucopyranoside, N-acetyl-D-glucosamine, and D-glucose.

7151 INSOLUBLE COLLAGEN OF THE GUERIN EPITHELIOMA.

(E.) Bankowski, E. (Acad. Med., Bialystok, Poland), A. Ratkiewicz, W. Galasinski and W. Rzeczycki. *Neoplasm* 21(3):285-290, 1974.

Guerin epithelioma cells were transplanted s.c. into Wistar rats and allowed to grow for 2-6 weeks. At intervals during this period, whole tumors were removed and homogenized and insoluble collagen prepared from them; collagen was also prepared from rat tail tendons. Tumors at different stages of growth contained hydroxyproline in concentrations which remained relatively constant in relation to nitrogen and protein. The tumor collagen showed a significant resistance to the dispersing action of EDTA, a resistance which increased with tumor growth. The insoluble collagen from the mature tumor was about eight times less dispersible by EDTA than the rat tendon collagen. The EDTA-resistant tumor collagen fraction contained 3.3% less nitrogen than the EDTA-resistant tendon collagen and small amounts of proline and hydroxyproline. During tumor growth, the hydroxyproline content increased significantly, while the proline content decreased slightly. The ERDA-resistant tumor collagen fraction contained more hexoses than the corresponding fraction of tendon collagen; in the mature tumor collagen the hexosamine content was almost identical with that of the corresponding fraction of tendon collagen. The hydroxyproline:nitrogen ratio in the tumor collagen was 4-5 times less than that in the tendon collagen. The insoluble tumor collagen did not swell in citrate buffer, pH 3.7, or dilute acetic acid. No gelatinization was observed during prolonged heating of the tumor collagen in acidic solutions.

7152 BLOOD GROUPS IN PATIENTS WITH MALIGNANT MELANOMA OF THE SKIN.

(E.) Ikonopisov, R. L. (Med. Acad., Sofia, Bulgaria) and T. I. Tsanov. *Tumori* 60(5):361-363, 1974.

The possible correlation of human skin cancer with blood group type was investigated in a survey of 414 cases of malignant skin melanoma, 862 cases of squamous cell skin carcinoma and statistical data from undiseased controls (Bulgarian population). The distribution of O, A and B and AB blood groups was 32.1%, 44.8%, 16.8% and 6.3% resp. For malignant melanoma it was 42.8%, 39.2%, 11.7% and 6.3%, and for skin carcinoma 34.5%, 43.5%, 14.8% and 7.2% resp. From statistical analyses of these distributions, it is concluded that skin carcinoma is not peculiar to any of the blood groups but the incidence of malignant melanoma is higher in type O blood than in the other types.

7153 SEX CHROMATIN IN ELDERLY WOMEN WITH MALIGNANT NEOPLASMS OF THE GENITALIA AND BREASTS.

(Ukr.) Voitenko, V. P. (Inst. Gerontol., U.S.S.R.). *Pediatr Akush Ginekol* 35(6):56-57, 1973.

The content of sex chromatin in epithelial cells of mucous membrane of the mouth in a group of 116 women (ages 60-79) with medically recognized malignant neoplasms of the genitalia (81 cases) and breasts (35 cases) in the early stages of their hospitalization was determined by Sanderson's method. Similar determination was performed for comparative purposes on two similar age groups: one group of 199 women (Control Group I) free of any malignant growth maladies, yet affected with various diseases connected with age pathology (arthritis, hypertension, diabetes, etc.), and another group of 261 women (Control Group II), examined by a special medical body and declared free of any maladies whatsoever. Results indicated that members of the cancerous group, when compared with members of Control Group I, more often showed either very low or very high frequency of sex chromatin. Yet there was no essential difference in the quantity of chromatin-positive nuclei between these two groups. On the other hand, the content of sex chromatin between members of Control Group II differed considerably, being significantly lower for members of Control Group II than for either the cancerous group or Control Group I. A large quantity of chromatin-positive nuclei in epithelial cells of the mucous membrane of the mouth might be considered as a nonspecific indicator for a tendency toward a number of diseases characteristic of pathological aging, especially for growth of malignant neoplasms of the genitalia and breasts.

7154 THE SURVIVAL VALUE OF THE DORMANT STATE IN NEOPLASTIC AND NORMAL CELL POPULATIONS.

(E.) Clarkson, B. D. (Mem. Sloan-Kettering Cancer Cent., New York, N.Y.). *Cold Spring Harbor Conf Cell Proliferation* 1:945-972, 1974.

Continuous ^{3}H-thymidine infusions and radioautographic analysis have shown that significant fractions of leukemic and other neoplastic populations can remain dormant (G_1 or G_2 phase) *in vivo* for several months at least. The dormant state is probably a major cause of therapeutic failure in acute lymphoblastic anemia and other diseases that are moderately responsive to chemotherapy. Dormant cells are completely resistant to agents, such as arabinosylcytosine, active against proliferating cells and are relatively insensitive to cell cycle phase nonspecific agents such as cyclophosphamide. Small dormant spontaneous ALR thymic leukemic lymphoblasts, when transplanted in young normal AKR mice, are capable of proliferating and causing death from leukemia at the same time as do large, actively proliferating cells. Thus, it is probable that the long-term dormant subpopulation contains many leukemic cells with stem cell capability and that all of them must be destroyed to effect a cure. The dormant state is not unique to neoplastic cells, but rather a property shared with their normal ancestors that greatly increases any population's probability of survival. *In vitro* studies of the survival value of the dormant state in human hematopoietic cells (Sk-L7) show that even in a stem cell population growing at its maximum rate with a doubling time of 16 hr, a tiny fraction of the population may remain dormant for a wk or more and then repopulate the culture. Over a limited range, the duration of survival is related to the total size of the population. When the population density increases and growth slows, the size of the dormant fraction and the duration of the dormant state increases, further enhancing the population's probability of survival.

7155 SURFACE MODIFICATION AND ELECTROPHORESIS OF NORMAL AND TRANSFORMED BHK21 CELLS. *(E.)*

Latner, A. L. (R. Victoria Infirm., Newcastle upon Tyne, England) and G. A. Turner. *J Cell Sci* 14(1): 203-214, 1974.

The electrophoretic mobility, surface modification, and the effect of histones, formaldehyde, and neuraminidase on a line of baby hamster kidney cells (BHK21/C13) were compared with malignant viral-transformed derivative cells (BHK21/C13/Py6). Cells were grown in media containing 80% Eagle's Medium (Dulbecco's Modification), 20% calf serum, glutamine, $NaHCO_3$, penicillin G, streptomycin sulfate, and mycostatin. Cells were washed and placed in an isotonic sucrose solution for electrophoresis at pH 7.5. The mean electrophoretic mobilities of these untreated Py6 and normal BHK21 cells were not significantly different. Treatment with 40% Analar formaldehyde caused a 4% increase in electrophoretic mobility of the BHK21 cells, but it caused a 22% increase in the Py6 cells (viral-transformed cells). Treatment with 10 U of neuraminidase/10^6 cells significantly reduced the mobility of both cell types, while only the Py6 cells showed a reduction (15%) in mobility when treated with neuraminidase followed by formaldehyde. The mobility in histone solutions (8-50 μg/ml) had no relationship to histone concentration. However, there was a high negative correlation (correlation coefficient -0.91) when the amount of histone per cell, calculated from total histone present divided by the total number of cells, was plotted against electrophoretic mobility. Over the range of 0-35 pg/cell histone, there was no change in mobility of the Py6 cells. At concentrations above 40 pg/cell, there was a rapid decrease in mobility; at about 50 pg/cell, the mobility was the same as that of the normal cells. These results indicate a difference between the surface membranes of normal and transformed BHK21 cells (with respect to sialic acid molecules) which did not affect electrophoretic mobility but which depended on a different distribution of charged groups.

7156 PHOSPHOLIPID COMPOSITION OF MITOCHONDRIA AND MICROSOMES FROM REGENERATING RAT LIVER AND HEPATOMAS OF DIFFERENT GROWTH RATE. *(E.)*

Bergelson, L. D. (Shemyakin Inst. Bioorganic Chem., Acad. Sci. U.S.S.R., Moscow), E. V. Dyatlovitskaya, I. B. Sorokina and N. P. Gorkova. *Biochim Biophys Acta* 360(3):361-365, 1974.

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Armelin, H. A. (Biol. Dep., Univ. California San Diego, La Jolla), K. Nishikawa and G. H. Sato. *Cold Spring Harbor Conf Cell Proliferation* 1:97-104, 1974.

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Jobsis, A. C. (Lab. Pathol., Wilhelmina Gasthuis, Amsterdam, Netherlands), J. F. M. Delemarre and P. A. Voute. *Maandschr Kindergeneeskd* 42(11/12):458-464, 1974.

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Tsuiki, S. (Res. Inst. Tuberc., Lepr. Cancer, Tohoku Univ., Sendai, Japan), T. Miyagi, K. Kikuchi and H. Kikuchi. *Proc Fourth Int Symp Princess Takamatsu Cancer Res Fund, Tokyo* pp. 235-249, 1974.

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LaBrosse, E. H. (Inst. Gustave-Roussy, Villejuif, France). *Maandschr Kindergeneesk* 42(9/10): 407-417, 1974.

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PREFACE

Carcinogenesis Abstracts is a publication of the National Cancer Institute. The journal serves as a vehicle through which current documentation of carcinogenesis research highlights are compiled, condensed, and disseminated on a regular basis. It represents an integral part of the Institute's program of fostering and supporting coordinated research into cancer etiology. Issues of *Carcinogenesis Abstracts* normally contain three-hundred abstracts and three-hundred citations (unaccompanied by corresponding abstracts). Abstracts and citations refer to the current scientific literature that describes the most significant carcinogenesis research carried on at the National Cancer Institute, other governmental agencies, and private institutions. *Carcinogenesis Abstracts* is intended to be a highly useful current awareness tool for scientists engaged in carcinogenesis research or related areas. The great number and diversity of publications relevant to carcinogenesis make imperative the availability of this service to investigators whose work requires that they keep abreast with current developments in the field.

Carcinogenesis Abstracts is normally published monthly. Volume XII covers the scientific literature published from Jan 1974 through Dec 1974. To increase the usefulness of *Carcinogenesis Abstracts,* Volume XII, a Wiswesser Line Notation index and a Chemical Abstracts Service Registry Number index have been provided. These indexes reference compounds described in abstracted articles. A cumulative subject, author, CAS Registry Number, and Wiswesser Line Notation index for Volume XII will be published shortly after the final regular issue.

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NOTE

Journal names are abbreviated according to the list of abbreviations used by *Index Medicus*. For journals not covered by *Index Medicus*, the abbreviations (with some modifications) found in *World Medical Periodicals*, 3rd Edition, are used.

LANGUAGE ABBREVIATIONS

Afr.	Afrikaans	It.	Italian
Ar.	Arabic	Jap.	Japanese
Bul.	Bulgarian	Kor.	Korean
Ch.	Chinese	Latv.	Latvian
Cz.	Czech	Lith.	Lithuanian
Dan.	Danish	Nor.	Norwegian
Dut.	Dutch	Pol.	Polish
E.	English	Por.	Portuguese
Eston.	Estonian	Rum.	Rumanian
Fin.	Finnish	Rus.	Russian
Fl.	Flemish	Ser.	Serbo-Croatian
Fr.	French	Sl.	Slovak
Ger.	German	Sp.	Spanish
Gr.	Greek	Sw.	Swedish
Heb.	Hebrew	Th.	Thai
Hun.	Hungarian	Turk.	Turkish
Ic.	Icelandic	Uk.	Ukrainian
In.	Indonesian	Viet.	Vietnamese

ABBREVIATIONS USED IN ABSTRACTS

ACTH	adrenocorticotropic hormone	mg	milligram(s)
ADP	adenosine diphosphate	min	minute(s)
AMP	adenosine monophosphate	ml	milliliter(s)
ATP	adenosine triphosphate	mm	millimeter(s)
C	degrees centigrade	MTD	maximum tolerated dose
cm	centimeter(s)	ng	nanogram (10^{-9})
CNS	central nervous system	pg	picogram (10^{-12})
cpm	counts per minute	p.o.	orally
DNA	deoxyribonucleic acid	ppm	parts per million
e.g.	for example	r	Roentgen
g	gram(s)	RBC	red blood cells (erythrocytes), red blood count
μg	microgram(s)		
hr	hour(s)	resp.	respectively
i.m.	intramuscular	Rev.	review (only in citations)
i.p.	intraperitoneal	RNA	ribonucleic acid
IU	international unit(s)	s.c.	subcutaneous
i.v.	intravenous	sec	second(s)
kg	kilogram(s)	U	unit(s)
LD_{50}	median lethal dose(s)	UV	ultraviolet
m	meter(s)	WBC	white blood cells (leukocytes), white blood count
M	molar		
mEq	milliequivalent(s)	wk	week(s)
mM	millimolar	wt	weight(s)
μM	micromolar	yr	year(s)
mC,μC	milli-,microcurie(s)		

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51796	0059, 0093, 0127, 0360, 0632, 0657, 0665, 0706, 1281, 1287, 1827, 2883, 3032, 3097, 3099, 3100, 3733, 4203, 4266, 4299, 4801, 4803, 4870, 4904, 6001, 6621, 6729, 6730, 6731, 6893

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